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THE AMERICAN JOURNAL
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SEPTEMBER, 1943

ORIGINAL ARTICLES

FURTHER HISTORICAL AND EXPERIMENTAL STUDIES ON MENSTRUAL TOXIN*

By David I. Macht, M.D., F.A.C.P.
BALTIMORE, MD.

(From the Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc.)

Introduction. Nearly 20 years ago the present writer and Miss Lubin published their studies on menstrual toxin, which for the first time set forth scientifically controlled experimental data demonstrating the presence of a toxic substance—menotoxin—in the blood and other tissues of menstruating women.[82a,b] It is true that popular belief and folklore of all races throughout all time have ascribed a contaminating influence to women's touch during the menses but no experimental proof of such a poison has been handed down in the literature, and indeed with the rise of modern medicine the empirical taboos of older physicians and laymen have been derided as fantastic by scientists who never troubled themselves to make a disinterested scientific inquiry into the subject. We are therefore greatly indebted to Bela Schick for reviving the interest of the medical world in one of these superstitions, namely, the wilting of flowers handled by menstruating women, and for carrying out some tests on flowers they touched, the results of which suggested that there was even more contamination in such contact than superstition indicated. Professor Schick[120] admitted, however, that his experiments were not absolutely conclusive from the strictly scientific point of view. By a happy coincidence Macht and Livingston were working about that time in the laboratory for plant physiology at the Johns Hopkins University and engaged in the comparative study of the effects of various drugs and chemicals on living animal and plant tissues.[81] The results of these comparative experiments indicated at an early date that living plant physiologic test objects may be more sensitive to the deleterious effects of certain poisons than living animal tissues treated with the same poisons. Such an observation suggested an approach by phytopharmacologic methods to the search for small quantities of toxic substances in the blood and other secretions of animals and thus was discovered a new branch of biology to which the name of *phyto*pharmacology was

* Accepted for publication, January 29, 1943.

applied.[71n] In view of the interest aroused in the menstrual toxin by Schick's paper, an extensive phytopharmacologic research was begun on the blood and various secretions of menstruating women. Employing a rigidly standardized technique for studying the growth of *Lupinus albus* seedlings in hydroponic solutions containing different concentrations of salts and electrolytes essential for the perfect growth of plants under controlled conditions of light, temperature and other ecologic factors, the writer demonstrated that there is in the blood serum, blood plasma, saliva, sweat, tears, milk and other secretions of menstruating women a toxic substance which markedly inhibits root growth of *Lupinus albus* seedlings and produces deleterious effects on living plant protoplasm. Thus it was found that menstrual blood serum and saliva, unlike normal blood serum and saliva from the same women, definitely retarded root growth and stem growth and effected pathologic changes in protoplasmic streaming, respiration, transpiration and other physiologic functions of higher plants and also profoundly depressed the reproductive capacity of yeasts, fungi and various bacteria. Experiments on flowers were also performed by Macht and Lubin and although the results of such tests were not of themselves conclusive the definite acceleration of wilting observed in all corroborated the evidence obtained from quantitative studies on intact seedlings placed in hydroponic solutions. The data presented in the paper by Macht and Lubin gave strong support to many a popular empirical observation and the poisonous effect exerted by the sweat of menstruating women on living seeds and seedlings was very illuminative of certain injunctions found in the book of Leviticus[69] regarding the toxin of menstruation. These earlier phytopharmacologic studies on menotoxin also included experiments concerning the chemical nature of that poison. By the use of living seedlings it was possible to disprove the hypothesis certain investigators held concerning the chemical nature of menotoxin, *i. e.*, that the toxicity of menstrual serum and sweat was due to an excess of choline in those fluids. Macht and Lubin demonstrated that choline produced no injury of phytopharmacologic test objects. On the other hand, numerous experiments with various compounds thought to be chemically related to menotoxin led the writers to conclude that it was chemically closely related to *oxycholesterol*. This theory, as will be shown later, has been strikingly supported by the work of Rahn and his associates.

Ten years after the appearance of the Macht and Lubin paper, the senior author in collaboration with Miss Davis presented a second research on this subject in which he reported the results of further phytopharmacologic experiments on menstrual poison and also announced newer findings which had to do with the injurious effect of menotoxin on various animal tissues.[75] Thus it was shown that on smooth muscle preparations and particularly on the vas deferens of the white rat, menstrual serum exerted pharmacologic effects which differed qualitatively and quantitatively from those of normal human blood on the same test objects. It was found that menstrual serum produced in the vas deferens of the white rat a sensitization to epineph-

rine not unlike that effected in smooth muscle preparations for the same drug by applications of thyroxin.[4] Menstrual serum was also found to be toxic for paramecia and goldfish and the authors also described experiments on the sciatic nerves of rats which indicated that applications of menstrual serum killed the activity of these nerves sooner than did normal human blood serum in control experiments. Findings reported also by Macht and Hyndman[79a] revealed that injections of menstrual serum were very depressant for the behavior of rats trained to run in the circular maze. The paper of Macht and Davis, read before the Section on Psychology of the American Association for the Advancement of Science in 1932, and embodying new material and reviewing data published on this subject by foreign investigators, appears to have given some impetus to the study of menstrual toxin for a number of interesting and important experimental and clinical studies have appeared in European literature since that date. In the following paper the writer wishes to present the new experimental data concerning menotoxin gathered in the laboratory and to review the literature appearing on the subject in the past 10 years with a view to correlating the considerable store of scientific data concerning menstrual toxin which has been published with the older empirical information gleaned from folkloristic accounts on the subject and of discussing these data with regard to their significance in clinical medicine.

Experimental Psychologic Studies. Inasmuch as the most striking clinical symptoms in women at catamenia have to do with the nervous system, the writer, who has always been interested in psychopharmacologic effects of drugs—that is, their action on neurologic functions—undertook an extensive experimental investigation of the psychologic effects of menotoxin on albino rats. Specimens of menstrual serum were obtained from various individuals and after their toxicity had been established by phytopharmacologic tests on *Lupinus albus* seedlings, they were placed in the icebox, various doses being injected into rats and employed in psychologic tests. Three lines of investigation were followed in this research. In the first series of experiments the effect of normal serum and of menstrual serum injected into rats was studied on their behavior in the circular maze. The apparatus employed was a modification of the original Watson apparatus and is reproduced in Figure 1. Food was placed in the center of the maze and the rats were trained to thread the runways to the food in the shortest time possible without running into blind alleys. Once the animals have learned the maze their performance is remarkable for they run with clocklike precision by the shortest route, often in but 5 seconds from the periphery to the center of the apparatus, without a single mistake. Such trained rats are admirably adapted to the study of the effects of drugs or poisons on the neuromuscular behavior and what may be termed the higher psychologic functions of the animals and have often been employed by the author for that purpose.[73,85,87] Whatever theories are advanced concerning rats learning the maze problem, all authors agree that such a performance involves not only a response to propriosensitive stimuli but also a choice reaction or

discrimination.[12,54] The goal gradient, the food placed in the center of the maze, is one basis for the blind alley elimination, and the animal uses its sense of discrimination and judges the shortest path to the goal under the hunger drive. But the maze problem involves other factors which Woodworth describes in detail.[145] The rat learns its

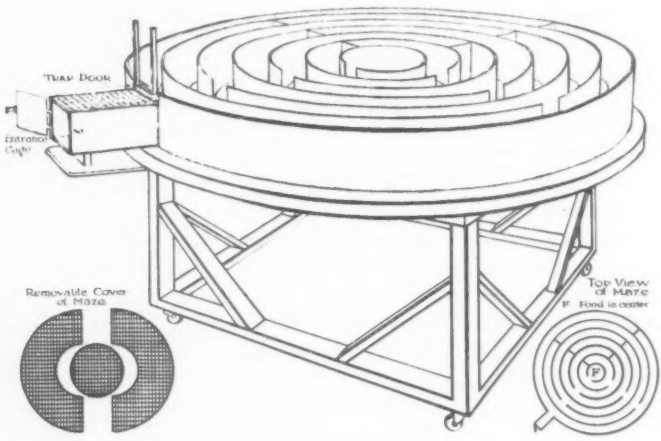

FIG. 1.—Circular maze.

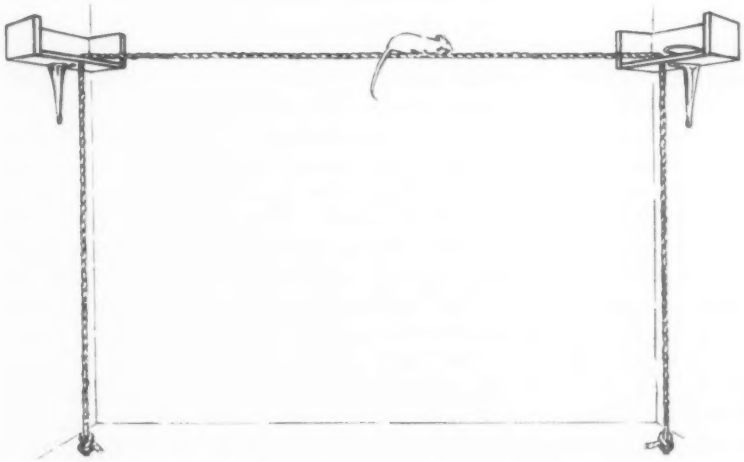

FIG. 2.—Test for neuromuscular coördination.

way from the periphery to the center of the maze by distinguishing objects in their spatial relations and in learning these in terms of a total pattern must exercise some function of higher nerve centers than those concerned with simple reflex responses.

A second series of psychologic experiments was made on rats trained to walk the tight rope, like the acrobat, from one platform to another

where food had been placed. This exercise involved a delicate neuromuscular coördination of the animals and after much training the rats learned to balance themselves very well and ran from one end of the rope to the other in very short time. Such a setup, illustrated by Figure 2, has been employed by Macht and Ulrich[88] for psychopharmacologic work.

A third series of experiments was made with rats trained to climb another 5-foot rope fastened to the floor at one end and to a platform holding food, on the other, an exercise involving neuromuscular coordination but especially designed for testing the ergographic or work

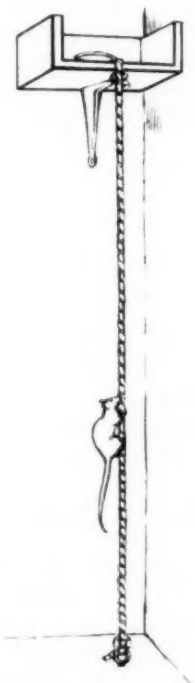

FIG. 3.—Test involving neuromuscular coördination and work capacity.

capacity of the skeletal muscles. The writer does not know of any previous published work in which such a test was employed. This setup is shown in Figure 3.

Experiments were made on 24 adult rats, 12 male and 12 female, injected on various days with normal human sera and with menotoxic sera. To avoid anaphylactic reactions, which are not very common in rats, the writer was careful not to repeat the serum injections after long intervals.

Table 1 shows the results obtained in 21 experiments. It will be seen that some animals were taught all 3 forms of exercise while others were used in but 1 or 2 of the tests. The effect of menotoxin

varied with the dosage and also with the toxicity of the sera determined by phytopharmacologic methods. There was no doubt about the poisonous nature of menstrual serum for a sufficiently large dose will kill a rat. Two such casualties, of a male and a female rat, respectively, are noted in the table, which does not show the results of other tests that the writer made in this connection. Small doses of menotoxic serum, as compared with the controls, profoundly depressed the neuromuscular system. It will be noted that such injections were particularly disturbing in the rope-walking and rope-climbing experiments, thus indicating the depressant action of this toxin on the muscles. In most cases also the activity of the rats in the maze was definitely impaired as the slower movements and longer running time of the animals as well as the occasional errors they made plainly indicated. These findings agree with those obtained by Macht, Hyndman and Davis. The toxicity of large doses of menotoxin for rats has recently been described also by Smith and Smith.[126]

TABLE 1.—RESULTS OF MENOTOXIN EXPERIMENTS ON ALBINO RATS

Exp. No.	Sex of rat	Amount of substance injected		Number of seconds animal required to: Run the maze Before	Run the maze After	Walk the rope Before	Walk the rope After	Climb the rope Before	Climb the rope After
				Injection		Injection		Injection	
1	F	Control: NaCl sol., 0.9%	1.0 cc.	6.3	6.0	6.3	5.0	8.0	13
2	F	Control: P.A. serum	0.5 "	6.0	7.6	4.6	5.0	9.0	13
3	F	Menotoxin serum (L)	0.25 "	6.0	25.0[2]	5.3	9.0[2*]	14.0	Stalled
4	F	Monotoxin serum (L)	0.35 "	5.6	..	6.6	..	10.3	Died
5	F	Control: normal serum	0.3 "	5.6	5.0 6.0				
6	F	Control: normal serum	0.3 "	11.0	8.6 7.3				
7	F	Control: pemphigus serum	0.5 "	6.0	5.0				
8	F	Menotoxin serum (S)	0.1 "	6.0	6.3				
9	F	Menotoxin serum (S)	0.2 "	6.3	8.0				
10	F	Menotoxin serum (S)	0.3 "	6.0	8.0				
11	M	Control: normal serum	0.4 "	5.6	5.0	5.6	5.6		
12	M	Control; normal serum	0.75 "	6.6	6.6				
13	M	Menotoxin serum (S)	0.2 "	5.0	6.0				
14	M	Menotoxin serum (S)	0.3 "	5.3	6.6	6.0	5.6		
15	M	Menotoxin serum (X)	0.4 "	6.3	Stalled	..	..	6.6	Stalled
16	M	Menotoxin serum (X)	0.3 "	6.6	7.3				
17	M	Menotoxin serum (B)	0.5 "	8.0	19.0[2]	..	Stalled	8.1	Stalled Died
18	M	Control: normal serum	0.5 "	6.0	5.0				
19	M	Menotoxin serum (S)	0.5 "	6.0	8.0				
20	M	Menotoxin serum (S)	0.2 "	5.0	6.0	5.6	5.0		
21	M	Menotoxin serum (S)	0.3 "	6.0	8.0 25.0[2] 19.0[2]	6.3	7.0		

* Superimposed figures indicate number of errors.

Absorption of Poisons Through the Male Genitalia. In order to determine whether or not menstrual poison can exert a deleterious effect on the human male or, more specifically, on the mates of menstruating women, the writer began a series of experiments dealing with the broader question of possible absorption of drugs and poisons through the male genitalia. The method of approach was like that which he used for many years in studying absorption of drugs through other unusual channels such as the eye,[71c] ear[71f] urethra,[71d] esophagus,[71] vagina,[71e] and skin.[71g,r] Experiments were made on guinea pigs; rabbits and cats by applying solutions of powerful pharmacologic agents

to the prepuce and penis and observing the symptoms of systemic absorption (if there were any) and effects on various physiologic functions. The striking results obtained demonstrated that many drugs and poisons are readily absorbed through the male organs. These experiments will be described in detail in a journal devoted to genitourinary diseases. It will suffice to state here that the writer found that rapid absorption followed such application of the antiseptics phenol and cresol, of nitroglycerin, of the alkaloids atropine, strychnine, aconitine and nicotine, of the volatile oils and also of cobra venom. Thus the rapid absorption of aconitine or a minute quantity of nicotine alkaloid, applied in this way, was demonstrated by the characteristic effects on the respiration and blood pressure curves traced on a kymograph. Such administration of these powerful poisons was followed by death in a few minutes. Applications of strychnine to guinea pigs elicited typical convulsions. Similar use of atropine and pilocarpine was succeeded by the characteristic pharmacologic effect, indicating their absorption into the circulation.

It was more difficult to determine whether or not menstrual toxin may be absorbed through the male genitalia. Experiments were made on rabbits and cats under general anesthesia. In such animals the effect of normal human blood serum introduced into the preputial sac was compared with that of menstrual blood serum similarly administered. In some of these tests, but not in all, it was found that menstrual serum differed from the control in its effect on the blood pressure. The menotoxic serum effected a definite rise in blood pressure while normal serum had little or no effect on the curve (see Fig. 4). While it cannot be stated with absolute certainty that the rise in blood pressure was due to absorption and the consequent vasoconstricting effect of menotoxin, this may be assumed in view of Labhardt and Hüssy's[65] studies on the vasoconstricting property of menotoxin on peripheral vessels.

To ascertain more definitely whether the absorption of blood serum in general may be accomplished by its local application to the prepuce or penis, the writer resorted to a new and very delicate biologic test, of immunologic character. Some years ago it was demonstrated that guinea pigs may be sensitized to horse serum[71k] and other proteins[71l] applied to the mucous membrane of the vagina. Such guinea pigs, treated locally with horse serum for several days and allowed to rest for a period of 2 to 3 weeks, developed typical anaphylactic or allergic phenomena when injected with a dose of the antigen after the rest interval. A similar procedure was employed in the present investigation. For 5 days male guinea pigs of various sizes were sensitized by daily applications of 0.2 cc. of horse serum to the prepuce or phallus. They were then allowed to rest for 2 or 3 weeks after which 1 cc. of horse serum was injected into the circulation of each. The marked allergic reactions elicited by such injections were identically like those exhibited by control guinea pigs sensitized 3 weeks before by single *subcutaneous* injections of horse serum. On the other hand, after large doses of the antigen had been injected directly into the circulation of

each of another series of control guinea pigs, not previously injected with horse serum, no allergic symptom, much less anaphylactic shock was noted.

In the present study experiments were made with a series of 30 guinea pigs. Of 10 which had been sensitized by subcutaneous injection of horse serum, all developed severe anaphylactic shock and 8 died. Ten other guinea pigs were sensitized by applications of horse serum to the male genitalia. Later, when the antigen was injected directly into the

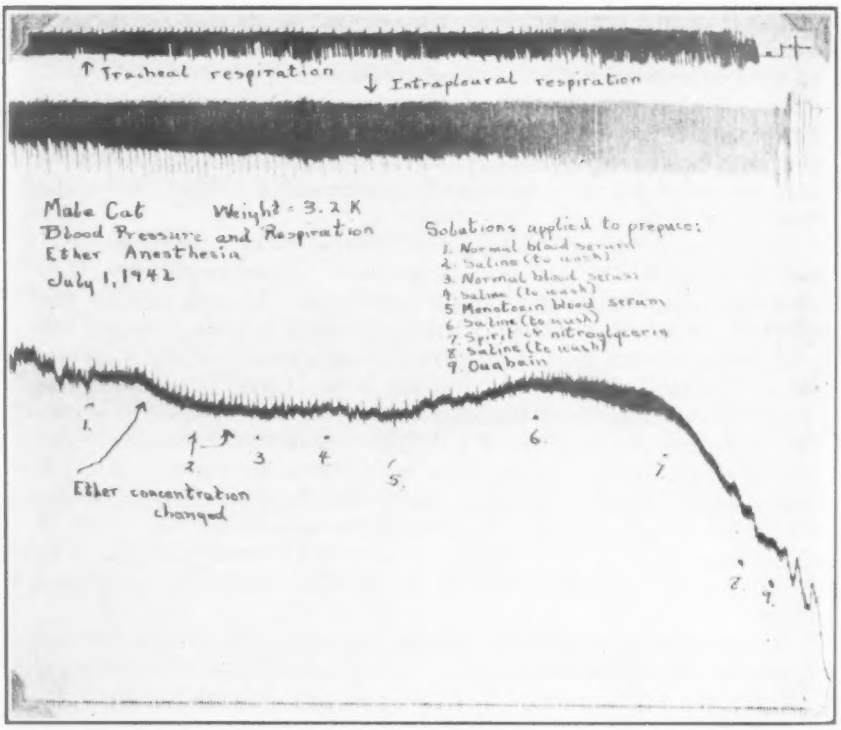

FIG. 4.—Effect produced on blood pressure and respiration by various substances applied to the prepuce of male cat under ether anesthesia. Note, at *3*, the negative effect of application of normal human blood serum; at *5*, the pressor action effected by menstrual blood serum; at *7*, the marked fall in blood pressure induced by application of dilute spirits of nitroglycerin; and, at *9*, the lethal effect of ouabain.

circulation, they developed violent anaphylactic shock. Seven of these animals died in from 5 to 10 minutes, while 2 exhibiting typical allergic reactions and 1 but mild allergic reactions subsequently recovered. Horse serum was injected directly into the circulation of the 10 guinea pigs remaining in the series, which were used as controls, and none of them displayed any sign of allergic reaction or anaphylactic shock. The anaphylactic shock developed in the guinea pigs treated with applications of the antigen to the genitalia was striking and characteristic. On its injection into the circulation of the respective

guinea pigs, they first became restless, then rubbed their noses and made clucking noises suggestive of respiratory obstruction. Auscultation of such animals with a stethoscope revealed marked bronchial constriction and squeaking râles. The animals began to tremble and shiver, then developed more or less violent convulsions, fell over on the side prostrated, passed into a comatose state and died. Postmortem examination usually revealed greatly distended lungs with constricted bronchi.

Such experiments as those described above are conclusive proof that sufficient blood serum can be absorbed through the prepuce or surface of the penis to sensitize the animals. If that is the case with normal blood serum, it may be assumed that similar absorption would follow applications of menstrual serum to the same organs; and if such a serum has a toxic property not exhibited by normal serum, it may be expected that it will exert a harmful effect on the animals. In addition, experiments were made on previously circumcised guinea pigs. Sensitization in these animals was also achieved by local applications of horse serum but the reactions after injection of the antigen were not quite so violent and suggested that absorption through the cornified integument was slower than it was through the mucous membranes of the uncircumcised animals.

It is interesting to note that historical and ethnologic studies inform us that among most peoples of ancient times as well as of the present day, cohabitation of husband and wife at time of catamenia is discouraged for either hygienic or esthetic reasons. Among the older nations we even find religious prohibitions to that effect, and every Bible student knows that the Old Testament enjoins complete separation of husband and wife not only during the period of actual catamenia but for 7 days after cessation of the flow. This empirical injunction is in obvious conformity with the findings made in the researches described above. Furthermore, we may observe that the ancient custom is in complete accord with the latest embryologic and endocrinologic teachings concerning the relation between menstruation and ovulation.[99b] The two physiologic processes are not synchronous. On the contrary, we now know that ovulation and fertilization of the ovum cannot take place at the time of catamenia and is accomplished in the intermenstrual interval. It appears therefore that the Hebrew prohibitions concerning menstruation have a sound embryologic and physiologic basis,[122] for the primary purpose of marriage according to all civilized religions is the propagation of the species.

Review of Experimental Literature. In order to examine more intelligently and critically the mass of folklore which has accumulated concerning menstruation and to secure a scientific basis for study of the periodic spells of malaise and illness from which women suffer, let us review the experimental evidence which has been amassed regarding the toxin of catamenia. In this way we shall sift the chaff from the wheat and discover the modicum of truth beneath the crust of myth, magic, poetry and superstition, and thus make a rational approach to the clinical problems involved in the subject. The experimental evi-

dence on menotoxin may be classified as: (1) botanical, (2) zoöpharmacologic, (3) psychologic, (4) physical and (5) chemical.

1. *Botanical Experiments.* The earliest work on effect of menotoxin may be dated from the investigation Bela Schick carried out on cut flowers. Such tests on flowers, also made by Macht and Lubin, by Frank,[40] Sänger[116] and others, cannot *per se* be regarded as conclusive scientific evidence of a menotoxin but they were corroborated by the more quantitative and controlled experimentation attempted later and they also served to enlist the interest of the medical world in the possibility of the existence of a menstrual toxin. The evidence derived from the tests made on cut flowers was inconclusive by reason of the inadequate methods employed for such study. Schick's tests on cut flowers might be roughly compared with experiments physiologists might undertake on decerebrated animals without ligating the blood vessels to maintain the circulation or making any provision for keeping the respiratory function intact. Experiments on cut flowers without proper plant-physiologic technique may be compared, too, to studies on surviving isolated animal tissues suspended in water instead of warm oxygenated physiologic saline solution. Quantitative plant-physiologic work on menotoxin may be dated from the experiments of Macht and Lubin on intact seedlings grown in plant-physiologic solutions of different chemical composition under standardized ecologic conditions. Employing such a method these authors demonstrated conclusively the presence of a toxic substance in the blood, saliva, tears, sweat, milk, urine and other secretions of women at catamenia, a toxin not found in the blood and other secretions of the same individuals during the intermenstrual period. Macht and Grumbein[78] demonstrated the presence of this toxin even in dry and frozen blood specimens, and Böhmer[11] duplicated their research on dry blood with a view to proving its usefulness for diagnostic purposes in forensic cases. But the studies of Macht and Lubin on root growth of *Lupinus albus* seedlings did not supply the only botanical proof they obtained of the existence of a toxin in menstrual blood and secretions. These authors have shown that menstrual serum, as compared with controls of normal human blood, exerted a toxic action on protoplasmic streaming, on germination of seeds, on growth of flowers and stems, on the geotropic functions of higher plants and on the respiration and transpiration phenomena of leaves. For practical purposes, however, the study on root growth of *Lupinus albus* seedlings has remained the most convenient method for quantitative study of the toxicity of menstrual blood, and the writer and his associates have found the phytopharmacologic method originally employed for this purpose equally useful for the detection of minute quantities of other toxins in several important clinical conditions.[77] Thus the phytopharmacologic method demonstrated the presence of a toxin in the blood of pernicious anemia,[71i,j,72] which is not found in other forms of anemia, namely, those due to malignant disease, tuberculosis, leukemia, and so forth. Similarly a toxin was discovered in the blood of leprosy,[71m] and an extensive investigation carried on by Macht and Pels furnished the first experimental

proof of the presence of a toxin in the blood of pemphigus.[86,103a,103b] Employing the phytopharmacologic method, the Russian pharmacologist Tscherkes was the first to call attention to the toxin of trachoma.[71o] Later his findings were confirmed by Macht on blood sera obtained from North American Indians suffering from the same disease.[71p] Further search for toxic substances specific for special diseases is now in progress in this laboratory. An important phase of such experimentation is that undertaken with blood sera from different types of psychoses.[84] A detailed description of all these findings does not fall within the scope of the present paper but it may be asserted that, having weathered 20 years of testing and criticism, the phytopharmacologic technique in competent hands yields quantitative experimental data proving reliable on statistical analyses[83] and often more dependable than the data obtained by zoöpharmacologic tests because of the inherent restrictions as to the number of available animals in an ordinary pharmacologic laboratory.

The use of living seedlings for the study of menstrual toxin seems to have received fresh stimulus since 1932, the approximate date on which newer phytopharmacologic as well as zoöpharmacologic studies on the subject were announced by Macht and Davis before the Section on Psychology of the American Association for the Advancement of Science. It is curious that some of the most scholarly researches on the subject have been conducted by pediatricians interested in investigating that toxicity of mother's milk during menstruation which was first demonstrated by Macht and Lubin. It is a well-established empirical observation among general practitioners as well as nursing mothers, and others, that human milk at catamenia is liable to upset the gastro-intestinal system of nursing infants. Accordingly, the matter was submitted to experimental study. Of special interest is the work of Fraenkel,[38] Silber,[124] Eltz,[31] Borsarelli,[13] Steinert and Papp[128] on the toxicity of human milk at catamenia by the phytopharmacologic method. Among the work published on this subject were the researches of Eltz and Mommsen,[95] who not only demonstrated the toxicity of menstrual milk for living seedlings but succeeded in obtaining toxic blood serum from rabbits fed with such milk.

A number of investigators have studied the effects of menstrual toxin on yeasts and bacteria. Macht and Hill have already reported experiments regarding the inhibitory action of menstrual serum, as compared with normal serum, on cultures of *B. coli*. Sutterlin and Szelinski[129] studied the effect of menstrual toxin on the keeping qualities of food. Christiansen[20] studied the toxic action of menstrual blood on *Lactobacillus bulgaricus*. Polano and Dietl[106] denied that menstrual toxin inhibited growth of yeast cells but their work has been controverted by numerous other experimenters who agree as to the toxic action of this substance on reproduction of yeasts and other fungi. Rahn[6,110] and his co-worker published elaborate quantitative studies concerning the inhibitory action of menotoxin on *Saccharomyces mycoderma*. Mommsen reported elaborate studies not only on poisonous effect of menotoxin on yeasts but also on the chemical nature of this toxic

substance, studies which refuted the so-called acetyl-choline theory concerning its structure. Böhmer showed that solutions of dry menstrual blood were toxic for yeasts as well as other plant-physiologic test objects, whereas the dry blood from control subjects exerted no such inhibitory action. Borsarelli found that menstrual toxin is toxic for the fungus *Aspergillus niger*. In this connection the empirical observations of a British physician, Burgess,[16] dating as far back as 1878, are of special interest, "It is a fact generally known to every housewife and cook that meat would spoil if salted at the menstrual period" and "It is undoubtedly the fact that meat will be tainted if cured by women at the catamenial period, and that hog meat will not cure or take salt when the rubber is menstruating."

2. *Zoöpharmacologic Experiments.* In the study of blood toxins in general and of menstrual toxin in particular, the phytopharmacologic technique yields more specific and hence more impressive results than zoöpharmacologic methods. Generally speaking, it has been found that substances toxic enough to injure living plants occur in the blood of but few pathologic conditions, which does not mean that menstrual toxin has no harmful effect on animal test objects, although it is not so specific and its action can be duplicated by many other poisons. Macht and Lubin found that menstrual serum is more toxic than normal blood for goldfish and paramecia. Macht and Davis reported other zoöpharmacologic findings. Menotoxin was found to effect such sensitization of vasa deferentia of rats as to make them abnormally responsive to treatment with epinephrine and ephedrine, a sensitization not unlike that Ascher noted after treatment of smooth muscle organs with thyroxin. Lanczos[66] studied the effect of menstrual poison on gastrocnemius preparations. Macht and Elvers[76] observed its action on living spermatozoa. Borsarelli found menstrual serum exceedingly poisonous for larvæ of *Bufo vulgaris*. Macht and Davis noted the toxicity of menstrual serum for rats, Smith and Smith later confirming and extending this finding. Strangely enough, Macht found that menstrual blood is less poisonous for mice than for rats but Levinson[68] discovered that it is very toxic for guinea pigs. Labhardt[64] and Hüssy[65] found that, as compared with normal blood, menstrual blood is rich in vasoconstricting substances which could be demonstrated by perfusion experiments and tests on surviving muscle preparations. Patzschke and Sieburg found that the sweat of menstruating women, as compared with that of the same individuals during the intermenstrual period, stimulated contractions of intestinal and other smooth muscle preparations. The present writer has already described the vasoconstricting effect of local application of menstrual serum to genitalia of male cats and rabbits. Eltz and Mommsen demonstrated the toxicity of blood of rabbits fed with menstrual milk. Psychologic experiments will be described below. Special mention, however, should be made of the work of various scientists on the coagulation of blood. Barthelmez's review of the subject contains a complete résumé of these findings. Studies have been made in particular by Zondek,[147] Kross,[63] Dogliotti[57] and Macht.[71a] All these investigators found that the coagulability of

menstrual blood differs from that of normal blood, the majority claiming that coagulation is delayed during catamenia.

3. *Psychologic Experiments.* It is a time-honored tradition that women at the time of their periods are "unwell," particularly in the psychologic sense of the word. Depression, as well as other nervous ailments, at that time are very common, but no experimental psychologic work of conclusive character has been published on the subject until recently. An objective experimental study on animals was first undertaken by Macht and Hyndman who studied the effects of normal and menstrual blood, respectively, on the behavior of albino rats in the circular maze. This research was repeated and extended by Macht and Davis. In the current study the psychologic experimentation already described has been carried on along three different lines of research. All the data yielded by such tests indicated that injections of menstrual blood sera profoundly affect the neuromuscular system of albino rats, and conform with the results of clinical studies made by various gynecologists and psychiatrists. Thus Tuttle[135] made a statistical study of the irritability of women during the menstrual cycle.

4. *Physical Experiments.* These have been concerned chiefly with radiologic studies. The subject of menstrual toxins is so intimately connected with numerous superstitions, folklore and taboos that the average investigator is inclined to lose his poise and impartial attitude when studying current experimental findings and to frown upon the work of the most reputable researchers. With this preliminary comment the writer proceeds to describe the remarkable experiments reported by Christiansen and by Rahn[109] and his associates, Barnes and Ferguson.[35] Interested in mitogenetic rays and the radiations given off by living cells, particularly of the human body, these investigators have reported the results of their quantitative experiments on yeasts. In a paper entitled "Das Menotoxinproblem und die mitogenetischen Strahlen," Christiansen showed that proliferation of yeast cells can be inhibited by emanations or radiations from the bodies of menstruating women having no other contact with the yeast cells. Rahn and Barnes and Ferguson published detailed reports of such studies, carried out on a larger scale. The Rahn school found that certain radiations of the human body, especially at catamenia, and occasionally also in course of certain diseases, definitely inhibited the growth of yeasts. Various parts of the body, such as the fingertips, and even the eyes were said to give off these radiations. The present writer did not succeed in confirming these findings phytopharmacologically; however, Rahn and his associates admit that there are wide individual variations with regard to lethal dosage, some giving off more emanations than others. The subject must be left *sub judice*, and the writer confines himself merely to quoting the statement of Rahn and Barnes published in 1933: "The old 'superstition' that menstruating women cause flowers to wilt and fermentations to become abnormal has been verified experimentally by several authors. In 1929, Christensen proved that menstrual blood will kill yeast cells, or change them morphologically, even through a quartz coverglass.

"The authors observed that this influence is not limited to menstruating women. One woman could not obtain growth of a very sensitive species of yeast for periods of 10 to 20 days, alternating with periods of normal growth. Radiation from her fingertips through a quartz plate killed the yeast in 15 minutes. One man killed yeast by this method in half of all tests. Even his eye and nose, at very close range through quartz, had the same effect. Since this radiation does not pass through glass slides, it is probably ultraviolet.

"Observations for more than one year suggest that this radiation is due to pathological conditions. Both the above cases can be explained in this way, and there are other similar observations.

"Macht and Lubin have shown the menstrual toxin to be identical with or closely related to oxycholesterol. We observed this compound to kill yeast through quartz. So did the ether washings from the fingers of menstruating persons. This kind of radiation may therefore be explained simply by a pathological excretion of oxycholesterol through the skin."

Here we may mention also the interesting work of Burr and Musselman[17] on the bioelectric correlates of the menstrual cycle, a research which does not deal with such ultraviolet radiations as engaged the attention of Rahn and his co-workers but with electric potentials in women before and during menstruation. These investigators found that the electric potential difference rises significantly during the days immediately following the menstrual period. There appears to be some connection between the menstrual cycle and certain electric potentials of the body tissues.

5. *Chemical Experiments.* While the chemical nature of menstrual poison is not yet completely understood and crystalline or chemically pure menotoxin has not yet been isolated, the experimental, pharmacologic and biochemical work already done throws considerable light on this subject. Labhardt and Hüssy concluded from their biologic experiments that menotoxin is a vasoconstricting substance. Sieburg and Patzschke,[123] Klaus[62] and others believed this toxin to be closely related to, if not identical with, choline. The phytopharmacologic findings of Macht and his collaborators contradict this hypothesis for they found that choline and acetyl-choline are not toxic for plant protoplasm. Ashley Montagu[96] believes this toxin to be identical with di-methylamine, a substance discovered, many years before, by Michin.[94] Macaggi and Sivori[70] regard it as closely related to thyroxin or the active principle of the thyroid gland. The curious findings obtained by Macht and Davis in their studies with menotoxin on the irritability of the vas deferens lend support to the latter view. Gautier[41] and Bourcet[14] also incline to the opinion that there is a chemical link between the arsenic and iodine content of the thyroid gland and the menstrual cycle. Steinert and Papp attribute the toxicity of human menstrual milk to a reduction in diastatic ferments. None of these hypotheses is supported by decisive chemical data and the majority of physiologic proofs adduced in their favor would not withstand a critical phytopharmacologic test, which seems to be more specific than

the zoöpharmacologic method in detecting menstrual toxin. The original findings of Macht and Lubin, based on such pharmacologic and biochemical experimentation, appear to indicate a closer relationship of menotoxin and cholesterin or, more strictly speaking, oxycholesterin, in chemical composition. Thus it was discovered that wherever found in the human body, whether in the skin fat or in the vernix caseosa or elsewhere, even weak solutions of oxycholesterin in plant-physiologic media markedly inhibited the growth of *Lupinus albus* seedlings. Unna and Golodetz[136] have shown that the human skin is rich in fats containing considerable quantities of cholesterol and cholesterol derivatives. Furthermore comedones and sebaceous glands of the face and fingernails have proved to be rich in cholesterol derivatives. Phytopharmacologic experiments performed by the writer revealed that extracts of all these were toxic for *Lupinus albus* seedlings. Other poisons of animal origin, such as the toad poison, *bufagin*, of Abel and Macht,[1] had been found by the latter to be very poisonous for plant growth. This toad poison, as well as others later studied by Chen,[19] are all derivatives of cholesterol. Perhaps the most striking single experimental proof supporting the original oxycholesterol theory was the finding by Rahn that inhibitory effects on yeast similar to those he had observed in his radiologic tests on menstruating women were produced by cholesterol itself. Further support is lent the cholesterol or oxycholesterol hypothesis by the fact that recent chemical studies on the structure of the sex hormones in general and of ovarian hormones in particular have disclosed their intimate relationship in chemical structure to cholesterin.[36] The findings made by Macht and his associates on the interrelation of menotoxin and bile salts also support this view.[79b] It is remarkable that such divergent substances as the carcinogenic chemicals, the digitaloid drugs, including toad poisons, the sex hormones, the group of vitamin D compounds and menotoxin are all derivatives of the phenanthrene nucleus and closely related to cholesterol.

Popular and Empirical Observations. The student of medical history will be amazed at the light shed by these accumulated experimental findings—botanical, zoölogic, psychologic, radiologic and chemical—on the numerous popular beliefs and taboos of the laity and empirical observations of the older physicians and naturalists concerning menstrual phenomena. Most of these empirical observations have a rational substratum. The wilting of flowers by the touch of menstruating women is a fact familiar to the majority of the sex the world over and what has long been styled superstition is now well attested by experimental scientific data. Even such extravagant remarks as those found in the writings of the elder Pliny[104] concerning the poison of menstruation and its contamination of seeds, garden plants, leaves and so forth, are now found to be not so far from truth as many pseudo-scientists would like to have it appear in their endeavor to disparage the keen observations of the older naturalists. The spoiling of such foods as fruit, vegetables and cucumbers and cabbages handled by menstruating women may be satisfactorily explained by the pharma-

cologic findings made concerning the effects of menotoxin on yeast, fungi and lower organisms. Similarly there appears to be some physical basis for the legal or systematic regulation in various industries prohibiting women who are menstruating from engaging in the manufacture of perfumes, tending of silkworms, gathering of mushrooms and expressing of grapes for the making of wine. The old British physician was correct in his inference that the curing of meats might not be successfully effected if they were handled by menstruating women. So we may also regard as needful the old Russian custom of discouraging the pickling of cucumbers or preparation of cabbage for sauerkraut by such individuals. In the light of the experimental findings of Macht and Lubin and also of pediatricians, we can now stress the fact that dough kneaded by menstruating women is liable to result in lumpy and poorly risen bread. A number of other popular observations concerning the poison of menstruation may be cited in this connection. The Frenchman, Laurent,[67] and the Englishman, Ellis,[30] tell us that in France women who are unwell are not admitted into those departments of sugar refineries in which the syrup is boiling and sugar is being bleached because it is believed that their touch would discolor the product. The same Laurent relates that in the Far East, menstruating women are not allowed to pick poppyheads and prepare opium because of the inferior product which would result. Were it not for the remarkable radiologic data published by Christiansen, Rahn, Barnes and Ferguson, the writer would refrain from quoting Laurent with regard to another and singular clinical observation. He described a series of cases of women musicians obliged to discontinue use of their stringed instruments during the menstrual period because of the effect of their touch on violins, violas, cellos and harps. The strings, it was said, did not emit the proper tone when manipulated by menstruating women and often snapped in the midst of their performance. While this last bit of narrative must be considered with reservation, it may be admitted that on the whole certain ludicrous aspects of some of the old taboos have possibly been exaggerated due to the superiority complex of certain professional modernistic folklorists with not sufficient insight to determine their origin. In the writer's opinion the remarks repeated above are not a whit more fantastic than the fatuous balderdash of such Freudian extremists as Gerson[42] or the maudlin vaporings of such professional mystical interpretations as emanate from Harding,[51] who writes on the man in the moon and the moon cycle of women as an astrologic phenomenon. The true scientist, who does not dwell on beliefs or unbeliefs but seeks for facts and truth, will certainly meditate on the extraordinary concordance of the Biblical account regarding menstruation and the phytopharmacologic and other experimental data on the subject accumulated in this paper. It may be well to add that even the puzzling references to postpuerperal phenomena in Leviticus XIII have also been found to have some basis in the findings of Macht and Leach[80] concerning the toxicity of puerperal bloods.

Interrelation of Menstruation and Various Physiologic and Pathologic Conditions. To assert that the menses affect a woman's well-being is

to state a platitude for the very expression "unwell" is universally descriptive of women at catamenia and implies a pathologic state of the individual. The profound physiologic changes occurring in the generative organs during the menses may also affect every other function of the female body. As modern physiology has developed, it has been found that numerous changes in functions of all the organs or parts of the female body, whether normal or diseased, run parallel with the menstrual cycle. Such studies were made over 70 years ago by the old authorities. Thus Schäffer[118] found that 74% of 220 healthy women complained of various subjective symptoms during their periods and 14% of these suffered to a pathologic degree. Similar studies were made by Töbler,[133] Rabuteau,[108] Goodmann,[43] Jacobi,[57] and others who stressed the wavelike change in metabolism and other functions which followed a course running parallel to the menstrual cycle. A host of later observers studied other physiologic phenomena in relation to menstruation. Kersh[60] and Reinl[111] measured the temperature. Ver Eecke[137] determined the output of urea, phosphates and chlorides; Wiessner,[143] Merletti,[93] Siredey,[125] and Francillion[39] noted the regularly recurring peaks and depressions in blood pressure which accompanied the menstrual period, and von Ott[102] published a significant work with graphs showing the intimate relation of fluctuations in temperature, pulse, blood pressure, lung capacity and various reflexes to the menses. Schräder[121] made biochemical studies on nitrogen elimination at that time. The newer literature on the interrelation of the menstrual cycle and women's normal physiologic functions is conveniently tabulated in Fluhmann's admirable monograph on "Menstrual Disorders."[37] Thus we find that a periodic rise in temperature has been observed by Cullis,[23] Jacoby,[58] and Rubenstein.[114] Such regularly recurring changes in blood pressure as have been reported by older clinicians have been confirmed by the work of Amos,[3] Griffith,[47] and Moore and Jenkins,[97] employing newer methods. Alterations in the pulse rate during the menses have been described by Truesdell and Croxford.[134] Quantitative biochemical studies on calcium content were made by Bock,[10] on potassium content by Spiegler,[127] on arsenic content by Guthmann and Grass,[49] on cholesterol content by Okey and Boyden[100] and Guillaumin and Vignes,[48] and on iodine content by Maurer,[91] and Scheringer,[119] and Hermstein.[53] Changes in the circulatory blood during menstruation have been followed by Holler, Melicher and Reiter,[56] and also by Dietre.[121] Hirsch and Hartmann[55] observed periodic increase in blood platelets during the menses while Eufinger[33] and Tedstrom and Wilson[131] noted regularly recurring fluctuations in the blood sugar irrespective of the diet. Changes in basal metabolism in relation to monthly periods have been studied by Benedict and Finn,[8] and also by Wakeham[139] and Wible.[142]

With regard to the relation between menstruation and various pathologic conditions, it is not surprising to find that even more striking changes have been observed by clinicians and correlated with the menstrual cycle as cause and effect. The course of various diseases may be influenced by the onset of catamenia and, conversely, many

maladies exert a marked effect on menstruation, some inducing menorrhagia and others amenorrhea. Interrelation of the menstrual cycle and various diseases was discussed 15 years ago by Aschner,[5] the eminent Viennese gynecologist, who inclined even then to the view that many pathologic conditions during catamenia may be precipitated or exaggerated by retention of menstrual toxin. A more recent account of the various diseases which may affect the menstrual cycle or which, conversely, may be affected by onset or suppression of menstruation will be found in the monograph of Emil Novak.[99a]

A host of pathologic symptoms and physiologic disturbances may either be precipitated or aggravated during the menses. Among the more important are the following:

1. *Disturbances of Neurologic and Psychologic Character.* These are among the commonest and range from the mildest forms of hyperirritability through the phobias, anxiety neuroses and depression to melancholia, mania and dementia præcox. Mary Chadwick,[18] in her monograph on "Psychological Effects of Menstruation," proposes that these psychologic disturbances in women at catamenia were the origin of the ancient prohibitions and limitations which later developed into all kinds of taboos. The insomnia, various types of psychologic neuroses and hysteria from which some women suffer periodically bear some relation to the menses. Epileptiform seizures are of greater violence or recur only at that time. Such studies on epilepsy have been made by Alexander,[2] Davidson,[24] and Gordon,[44] and Diepgen and Schröder[25] have investigated hysteria in relation to the menses. Various psychotic aspects of the subject have been investigated by Haymann,[52] MacKenzie,[89] Ross,[113] and Swanberg.[130] A case of acute insanity occurring regularly at catamenia has been described by the present writer.[71b]

2. *Headache.* Headache, often hemicranial, is a special form of neurosis which Aschner regards as an evidence of menstrual toxicosis. Scattered references to this symptom are found in the literature and a discussion of it appeared recently in the *Journal of the American Medical Association*.[107]

3. *Dermatoses.* Such manifestations are among the commonest noted in connection with catamenia. These skin lesions include furunculosis, acne, rosacea, herpes labialis, herpes zoster, urticaria, various forms of eczema, subcutaneous hemorrhage, erythema multiforme, erythema nodosum and localized patches of edema. Macht and Pels in their phytopharmacologic study of bloods from all kinds of dermatoses noted that the menstrual sera of women suffering from acne were generally more toxic than such sera from those who did not have acne. On the other hand, blood specimens from young men with widespread acne rarely exerted a phytotoxic reaction. Numerous dermatologic manifestations related to the menstrual period have been studied by Jerusalem,[59] Opel,[101] Edebohls[29] and others whose work is conveniently summarized in Novak's monograph.

4. *Ophthalmic Manifestations.* The laity have noted such symptoms from remotest antiquity and the baleful look of menstruating women receives frequent mention in the folklore. Organic disturbances

of the visual apparatus often occurring at catamenia include conjunctivitis, scleritis, iritis, herpes corneæ and retinitis, choroiditis and even detachment of the retina. Important contributions on the subject have been made by Dunn,[28] Greeff,[46] and Napier,[98] the last two authors writing on dysmenorrhea and amenorrhea.

5. *Arthralgias and Neuralgias.* All types of arthralgia and neuralgia are complained of during catamenia and may be precipitated periodically or may be exacerbated by onset of the menses. Aschner stated that of 700 patients whom he studied no less than 230 complained of varying degrees of arthritis and neuralgia. Some suffered from sciatica; others had inflammatory infections of the joints; and still others complained of lumbago and myalgias. All these ailments he attributed to excessive accumulation of menstrual poison in the system of menstruating women. More recently Rugh[115] described a series of arthritic cases which he also explained on the basis of the retention of the menotoxin in the system of his patients.

6. *Gastro-intestinal Manifestations.* Though usually of a mild character such manifestations may occasionally be of the more serious type described by Brush[15] and Plönies.[105]

7. *Hepatic and Cholecystic Manifestations.* These have also been noted during the menstrual period. Thus hyperemia and swelling of the liver have been described by Chvostek[21] and Fellner.[34] Periodic jaundice is mentioned by the famous German internist, Senator,[117] and gall bladder attacks in relation to the menses have been described by Binet.[9]

8. *Vasomotor and Other Disturbances of the Circulation.* Such manifestations during the periods have been described by Kisch,[61] Williams[144] and others.

9. *Thyroid Manifestations.* Mild periodic manifestations on the part of the thyroid gland are not uncommon during the menses but sometimes even the more serious forms of the disease, *i. e.*, exophthalmic goitre, are profoundly affected by the menstrual cycle. Studies on this subject have been published by Thoma,[132] Wallin,[141] and Woronytsch.[146]

10. *Respiratory Manifestations.* The respiratory organs have also been found to suffer in the course of menstrual abnormalities. All sorts of catarrhal conditions have been noted at catamenia. Asthmatic attacks have frequently been aggravated by onset of menstruation.

11. *Acute Infections.* Periodic menstrual manifestations of pathologic character have been observed in the course of various acute infections. Thus influenza has been studied in this respect by Osler and McCrae[102] and typhoid and pneumonia by Rosenstraus[112] and Esch.[32]

12. *Syphilitic Infections.* Of the chronic infections studied in relation to the monthly periods, syphilis has received the attention of Meirowsky.[92]

13. *Tuberculous Manifestations.* The most important chronic disease influenced by the menses and exerting a profound effect on the menstrual cycle is tuberculosis. Macht[71] studied this subject extensively in 1910 and it has also been investigated by Handford,[50] Gräfen-

burg[45] and others. There can be no doubt about the flaring up of acute symptoms in tuberculous patients at any time as manifested by rise in temperature, pulmonary hemorrhages and other pathological phenomena. The present writer noted a curious case of periodic intestinal hemorrhage in a patient with intestinal tuberculosis.[71a] The influence of chronic tuberculosis on the menstrual cycle has also been widely studied. In its early stages the disease may manifest itself by a menorrhagia whereas later it is usually accompanied by amenorrhea or complete suppression of the menses.

14. *Miscellaneous Manifestations.* Clinical investigators have correlated various other forms of pathologic disturbance with the menstrual cycle. These all attest the profound influence which the ovulatory cycle and catamenia exert upon practically every physiologic function of women. The cause of this periodic fluctuation in normal physiologic functions, including metabolism, and other pathologic symptoms appearing at the height of the menstrual period has engaged the attention of numerous students of medicine and is discussed below.

Discussion. A judicious and scientific appraisal of the experimental and clinical data presented in this paper justifies us in drawing several fairly definite conclusions. In the first place, the biologic experimental data demonstrate beyond reasonable doubt the presence in the blood and secretions of menstruating women of a toxic substance normally absent. Whether this substance be called toxin or abnormal product of metabolism or degradation product of an endocrine secretion is immaterial. Second, such a "menotoxin" provides a physical explanation of many curious popular beliefs and empirical observations of laymen handed down from remote antiquity. Third, abundant clinical evidence points to a periodic fluctuation in normal physiologic processes of women which runs parallel to the menstrual cycle. Fourth, numerous clinical observations have established the fact that many pathologic symptoms and objective signs of disease are correlated with the course of menstruation and abnormalities in the menstrual cycle. Two schools of investigators have tried to explain these pathologic manifestations, one of these, well represented by Aschner, asserting that the menstrual toxin plays the chief rôle in this connection, and the other, composed of many enthusiastic endocrinologists, contending that the female sex hormones cause most gynecologic ailments. Aschner's view gains some support from the fact that patients undergoing hysterectomy, even without removal of the ovaries, exhibit pathologic symptoms not attributable to lack of ovarian secretions and which may more logically be ascribed to chronic intoxication due to failure to excrete some toxic substance through the normal genital routes. The same is true of cases with ovarian transplants and substitution therapy. Aschner cites many cases of amenorrhea and scanty menstruation favoring the assumption that a menotoxin is thus retained by such patients. The symptoms observed in such cases are not unlike those noted in connection with dysfunction of and defective elimination of noxious products from other organs as in chronic dyspepsia,

constipation, impaired kidney function or secretion of the glands. Aschner claims that endocrine therapy in such cases is of no avail, while therapy aimed at promoting elimination of toxins and excretion of abnormal metabolic products often relieves the patients' sufferings. Although recent developments in the study of internal secretions have led to important discoveries, the therapeutic use of endocrines has not been remarkably fortunate, particularly in the field of sex hormones.

While all scientific investigation compels us to beware of drawing hasty, illogical conclusions and particularly of succumbing to the fallacy of *post hoc propter hoc*, the astonishing parallelism (revealed by newer quantitative pharmacologic measures), beginning a day or two before catamenia and reaching its peak at beginning of the flow, between menstrual toxemia and the exacerbations and remissions of clinical symptomatology and pathology, suggests a causal relation between the two phenomena. Results of the latest clinical studies can be best explained in this way. Thus Conn[22] describes a female patient with aggressive psychopathic personality who had exaggerated manifestations in the premenstrual period. The present writer also is acquainted with a case in which a young woman with psychopersonality shows extreme emotional instability and affective psychoses at that time. These acute exacerbations of mental phenomena conform with the phytopharmacologic findings Macht has made in such cases. Again, Waldbott and Bailey[140] have recently published a paper on estrogenic hormone determinations in premenstrual asthma, from which the impartial reader cannot discover whether the asthmatic attacks bore any relation to the hormone determinations or even that the endocrine injections definitely relieved the allergic symptoms.

The present writer holds that the distinction between the two views is largely academic and that the menstrual toxin and ovarian hormones are more closely related than has been assumed. In the female organism there is a periodic accumulation, under the influence of ovarian secretions, of certain substances essential to furtherance of fertilization and implantation of the ovum. Failure of this excretion, usually controlled by the menstrual flow, leads not only to retention of the poisons but to many pathologic symptoms. Recent chemical studies support this view for the findings of the present writer and his associates regarding the chemical structure of menotoxin and those of Rahn, Barnes, Ferguson and other investigators indicate its probable close relationship to oxycholesterol. It is now known that the ovarian hormones also are closely related to that substance, the investigations of Mandelshtam, Tschaikowsky and Bondarenko supplying further evidence[90] in this connection. Studying the chemical nature of menotoxin by phytopharmacologic methods, these investigators also experimented on the effect of folliculin and the lipoid portions of the ovaries on plant growth and concluded that menstrual poison is closely related to the ovarian sex hormones. The present writer found that while crystalline estrone solution has a tendency to stimulate root growth of *Lupinus albus* seedlings, crystalline progesterone definitely inhibits it.[74] The findings of Macht and his associates on the interrelation of meno-

toxin and bile salts also tend to support the view that menotoxin is closely allied to cholesterol. It is remarkable that such divergent substances as the carcinogenic chemicals, digitaloid drugs, toad poisons, sex hormones, ergosterol, calciferols and the group of compounds included under vitamin D, and menotoxin are all closely related chemically. All these are derivatives of phenanthrene and possess a structure not far removed from that of oxycholesterol. Such a relationship does not preclude the possibility of menotoxin's being much more toxic than the ovarian hormones as pharmacology abounds in striking examples of chemicals closely related in structure yet exhibiting diametrically opposite physiologic and toxicologic properties due to slight alterations in the relative position of some of their atoms.

Summary. 1. Experimental data demonstrate in the blood and secretions of menstruating women the presence of a toxic substance or menotoxin, which is poisonous for plants and animals.

2. This experimental biologic demonstration explains in large measure the numerous empirical observations on "menstrual poison" found in the folklore of many countries.

3. The interrelation of menstruation and various manifestations of disease is examined in the light of these findings.

4. Menotoxin has been found to be closely related pharmacologically to the phenanthrene derivatives, cholesterol and oxycholesterol; and, while it is a separate toxic entity, it is probably closely allied to the female sex hormones, which are also known to be natural products of phenanthrene and related in their structure to the sterols.

Addendum. After the above manuscript had gone to press, several additional items of interest appeared in the literature. Byrnes[17a] states that menstruating operators in certain industries stimulate corrosion of certain articles through contact with their perspiration. Hereford[32a] cites other instances of similar character. Basler[7a] by careful measurement found that the pull needed to epilate hairs was greater in the onset of catamenia than in the intermenstrual and the inefficiency of permanent hair wave manipulations at menses is discussed in a recent number of the *Journal of American Medical Association*.[107a]

The extraordinary concordance between recent findings about menstrual poison and the Biblical description on the subject gives support to the view that the Levitical ordinances concerning it, aside from their ethical, psychologic and religious significance, have a hygienic basis. In view of the newer advances in our knowledge concerning the chemical and biologic properties of water, the action of deuterium, of heavy water, and other isotopes, the specific characters of radioactive waters, the differences between ordinary and rain water, between natural and artificial sea water, etc., it would not be surprising to find a special hygienic significance in the elaborate ablutions recommended by the Scripture in connection with the postmenstrual purification rites.

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ACUTE INFECTIOUS POLYNEURITIS (GUILLAIN-BARRÉ SYNDROME)

A Brief Review of the Literature With Report of 3 Cases

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Since the publication by Guillain-Barré and Strohl[13] of a group of cases exhibiting a form of acute polyneuritis (or radiculoneuritis, as Barré called it), a good number of such reports of this disease in children have been published.[1,4–8,11,15,18,19] Casamajor and Alpert[4] collected the data on 19 cases in children, and added data on 3 children who had been studied by them. Since this latter report, De Sanctis and Green[8] have reported 2 additional cases, and in the excellent paper on this

disease by Roseman and Aring,[19] data on 4 children are included in a thorough study of 16 cases.

We have observed 3 children during a few months whose signs, symptoms and spinal fluid studies were such that we are reporting them as representing the picture described in the literature as Guillain-Barré syndrome (Tables 1 and 2).

TABLE 1.—SUMMARY OF DATA IN THREE CASES OF GUILLAIN-BARRÉ'S SYNDROME

Age (yrs.) Sex	Preceding illness	Initial symptoms	Findings in hospital	Course and comment
7-M	Cold 7 days; vomited once; irritable	Weakness of legs noted 2 days before adm.; could not stand day of adm.	Decreased power in arms, hands, thighs, feet, with hypotonia; deep reflexes absent in arms, very sl. in legs; cranial nerves intact; mod. nuchal rigidity; gen. muscular tenderness in arms and legs	Much impr. in 2 wks. after adm.; entirely recovered after 40 days; T.P.R. normal throughout hospital stay
7-F	Malaise 1 wk.; occ. vomiting 4 days; intermittent headache 3 days; loss of appetite 1 wk.	While in bed no muscle weakness noted; during first 4 days after adm. progressive weakness of legs, esp. of thighs	3 days after adm., lethargy, nuchal rigidity, bilat. periph. facial palsy, marked weakness of all muscles of legs, esp. thigh; consid. pain and tenderness in thigh and neck muscles	Impr. began 8 days after adm., but some nuchal rigidity for 4 wks. after adm.; able to walk 6 wks. after adm. and on discharge (2½ mos. after adm.) had regained most of her muscle power; no definite muscle atrophy; areflexia in legs still present
8-M	No preceding illness recorded; vomited twice 4 days ago	Pain and stiffness in knees for 4 days	Mod. nuchal rigidity; weakness of leg muscles, esp. thighs; triceps biceps reflex decr.; knee jerk, ankle jerk ab.; cranial nerves intact; severe pain in thigh and back muscles	Impr. rapidly, but complained of vague pains in shoulder, arms, legs occ. for 2 wks. after adm.; 31 days after adm. pt. had no complaints, was getting strong and able to walk; discharged 43 days after adm. with only a slight limp remaining

TABLE 2.—RESULTS OF EXAMINATION OF THE SPINAL FLUID

Date 1942	Cells	Protein (mg. per 100 cc.)
CASE 1		
1–15	2	54
1–19	6	112
1–22	1	115
1–30	2	qns
2– 6	..	67
2–24	2	46
CASE 2		
3–24	1	100
3–27	0	100
3–30	0	71
4–20	0	170
5– 6	..	qns
5–13	..	78
5–28	..	46
CASE 3		
4–28	0	60
4–29	0	qns
5– 6	..	..
5– 8	0	126
5–21	6	340
5–28	1	qns
7– 8	3	76

Discussion. These 3 cases had symptoms, physical findings and spinal fluid changes that probably justify their being classified as cases of Guillain-Barré's syndrome, known by many synonyms.*

The disease occurs most often in winter and spring. It shows no predilection for either sex, and apparently afflicts nearly all age groups, although none of the reported cases have been in children under 2 years of age. In the group of 19 cases collected from the French literature by Casamajor and Alpert[4] there were 9 cases under the age of 5 years, with 4 being 2 years old. So far, no etiologic agent has been proven as the cause of the disease.

In practically all of the reported cases the onset of the disease has been preceded by an upper respiratory infection. This latter infection in some cases preceded the disease by a week or more, and in others by a few days, or was present when paralysis occurred. Aches and pains are generally present, and these may be severe; tingling and burning may be annoying at times, while muscle tenderness is often marked. These sensory disturbances last for a varying length of time. One of our patients complained of pain on flexion of the neck for nearly a month; on the other hand, the sensory symptoms may last only a few hours, and occasionally the motor symptoms do not appear for days.

The weakness usually appears first in the distal muscles of the lower extremities, with a symmetrical involvement, which is valuable in diagnosis. Often within hours there is rapid involvement of muscle groups of the arms, with facial paralysis; and occasionally bladder and rectal sphincter loss. Cord involvement seems to be definitely less frequent in children than adults. This is true, also, of bulbar symptoms.

The changes in reflexes seem to be a rather constant finding, with all tendon reflexes in the involved limb absent, or diminished. Although facial paralysis is reported as being common, many of the children reported do not show it. From the total of 19 cases collected by Casamajor and Alpert,[4] and the 3 cases added by them, only 7 had facial paralysis, with another case being questionable. One of our cases had definite facial paralysis, and weakness of the right side of the tongue. Among the 16 cases studied by Roseman and Aring[19] there were 4 children, and 3 of these showed facial palsy. The paralysis when present is of the nuclear or infranuclear type, with involvement of the lower part of the jaw, brow and eyelid. Other cranial nerves than the seventh may be involved, but their involvement has not been reported as often in children as in adults.

There is mild systemic reaction as judged by the temperature and pulse rate. Most patients have either a slight fever or a normal temperature, with a normal, or only slightly increased leukocyte count. But the spinal fluid change is one of the most interesting and characteristic features of this disease. The typical spinal fluid finding is a normal cell count with a definitely increased protein content. Guillain-Barré

* Acute polyneuritis, infectious polyneuritis, motoneuritis, acute polyneuritis with facial diplegia, acute ascending paralysis.

and Strohl[13] in their original article stressed this finding, and Guillain[14] more recently has again emphasized the importance of albuminocytologic dissociation of cell count and albumin in the spinal fluid in the diagnosis of infectious polyneuritis. However, there are some practical points in this connection. The high protein content of the spinal fluid may not appear for a week or two after the patient becomes ill. Repeated taps may be required to find the change; but in some cases it may persist for years. It may be moderately increased or quite high, say, between 100 and 800 mg. per 100 cc. Unless the clinical picture is most typical, it would seem unwise to classify a disease as belonging to the one under discussion without a definitely increased spinal fluid protein, in the presence of a normal cell count. Quite a good number of the cases studied by Roseman and Aring and of those collected by Casamajor and Alpert had inconclusive spinal fluid studies. Repeated taps should be made as indicted. As has been emphasized,[8] similarity of this disease to acute poliomyelitis makes a diagnosis in the very early stage most difficult and important, and the spinal fluid picture is very helpful.

The pathologic picture has been studied by Holmes,[16] Bradford, Bashford, and Wilson,[2] Casamajor,[3] Viets,[21] Greenfield and Carmichael,[12] Sabin and Aring,[20] Honeyman,[17] and Roseman and Aring.[19] The report of the latter two writers is especially thorough and detailed. Microscopically, they report a moderate degree of edema of brain, spinal cord, and peripheral nerves. Acute passive congestion and toxic changes in the liver, kidneys, heart, and spleen; there was varying degrees of atelectasis of the lower lobes of the lungs, and an acute bronchitis. Microscopic studies of the nerves showed marked edema of the bundles, congestion, infiltration of inflammatory cells, swelling and beading of myelin sheaths, and fragmentation, beading and swelling of axis cylinders. The cord revealed some involvement of the cells of the gray columns in all cases, with the changes being most conspicuous at the cervical and thoracic level. Vacuoles were found in the cytoplasm of some of the anterior horn cells. Irregularity in the size of the axones was noted in the white matter of the cord.

The brain stem changes were rather similar to those of the spinal cord, while the cerebral hemisphere and cerebellum showed "outfall" of nerve cells, shrinkage of the ganglion cells, congestion, swelling of the oligodendroglia of the white matter and cellular infiltration of the meninges. Microscopically, studies of the viscera showed areas of degeneration in the adrenal cortex, interstitial infiltration of the heart, focal necrosis of liver cells with areas of focal degeneration; the kidneys showed collections of mononuclear cells, while in the spleen there was an extreme abundance of red cells.

The prognosis as to life is good in this disease, as it is seen in children, although the mortality is rather high among adults. In a series of 26 cases, of which 3 were children, reported by Forster, Brown and Merritt,[10] the mortality rate was 42%. Of the 3 children in their group, 2 died. However, of the 22 cases in Casamajor's and Alpert's report there was only 1 death. Both of De Sanctis' and Green's cases, the 4 children in Roseman's and Aring's series, and our 3 cases recovered.

The prognosis as to complete recovery of muscle function is more difficult to determine from the reported cases. In many instances the patients were not followed for a long period of time, or information is so recorded that one cannot be sure of the exact state of muscle function. "Gradual improvement" is often the final note made; or a patient may be described as being "well" when last seen. Of the 22 cases in Casamajor's and Alpert's report, there were 13 in which recovery seemed complete. It would seem, that while there is a good outlook for a return of normal muscle function, the improvement may take many months. The rapidity with which improvement begins seems to indicate a good prognosis. The chief ill-effects that are seen usually consist of weakness of the involved muscles with contractures.

Summary. Three cases are reported whose signs and symptoms and spinal fluid findings were such that they have been classified as belonging to the Guillain-Barré syndrome.

A brief discussion of the clinical picture has been presented.

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HYPERGLOBULINEMIA

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Almost invariably when the total protein concentration of blood serum is increased above normal this increase is because of an increase in serum globulin concentration.[15] A study of hyperproteinemia is,

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therefore, essentially a study of hyperglobulinemia. Furthermore, with three major exceptions,* whenever the globulin concentration is increased above normal the total protein concentration is usually also increased above normal.[15]

In 1937 Jeghers and Selesnick[10] reviewed the subject of hyperproteinemia and presented 13 cases due to 6 different causes. In this paper we shall present 50 cases due to 16 different causes. These 50 cases represent 9% of a series of 576 patients on whom 995 determinations of blood serum protein were done. The data were collected over a period of 3 years. The analyses were done by a macro-Kjeldahl modification of the Howe[8] procedure and the author's falling drop technic.[13]

Hyperglobulinemia is considered to exist when the serum globulin concentration is above 3 gm. per 100 cc. or when, in the absence of a globulin determination, the total protein concentration is above 8 gm.[14,15] Whenever the total protein concentration is above 7.5 gm., an abnormal increase in serum globulin is suspected but is considered to be certain only upon actual determination of the globulin concentration.

Of the total number of cases studied, 35 (6.1%) were found to have values for total protein concentration above 8 gm. Values between 7.5 and 8 gm. were found in only 20 patients out of the total of 576. Because in our study of 150 normal individuals[14] we found only 1 with a total protein concentration above 7.5 gm. (7.6 gm.), these 20 were considered as suspicious. However, only the 15 in which the serum globulin concentration was shown to be above normal were included in our statistical analysis. These 15 cases plus the 35 noted above comprise all the cases of hyperglobulinemia discussed below and constitute 9% of the total number of cases studied.

Bing and Plum[2] have presented clinical evidence which suggests that the origin of globulin is in the plasma cell of the bone marrow and in the reticulo-endothelial cells everywhere. Sabin[24] has reviewed immunologic and cytologic evidence which supports this theory. There also seems to be some relationship between globulin formation and the liver. An increase in serum globulin occurs in hepatic involvement of various kinds and is particularly common and marked in cirrhosis.[16] Aside from its rôle as antibody almost nothing is known of the functions of globulin. The evidence showing that the circulating antibodies are contained in the globulin fraction of serum has been recently reviewed.[23]

Diseases Involving Bone Marrow. The accompanying table shows the results obtained in diseases involving the bone marrow. The highest value for serum protein concentration obtained in this entire study was 10.1 gm.; this was in a case of monocytic leukemia which

* These three major exceptions are: (1) all forms of hepatic disease; (2) acute glomerulonephritis; and (3) the simultaneous occurrence of two conditions, the one causing hyperglobulinemia, the other causing hypoalbuminemia. These conditions have been discussed in an earlier report and will not be considered here.[15] In these instances the globulin concentration is frequently increased but the albumin concentration is at the same time frequently decreased so that the total protein concentration does not always increase when the globulin is increased.

was proven by autopsy. The patient had a serum globulin concentration varying between 4.3 and 6.7 gm. This case is of considerable interest because the monocyte is generally considered to be part of the reticulo-endothelial system. Should such marked increases of serum globulin be found to be usual in this disease, it would lend further support to the theory that globulin is formed by the reticulo-endothelial system.

In our 1 case of lymphatic leukemia there was an increase in serum globulin. There are reports in the literature of increased serum globulin in 2 cases of myeloid[7,12] and 1 of lymphatic leukemia,[11] but the incidence is not known. In neither lymphatic nor myeloid leukemia, however, are the increases nearly as striking as in the case of monocytic leukemia cited above.

The association of hyperproteinemia with multiple myeloma was probably first shown in 1917 by Jacobsen.[9] The highest serum protein concentrations reported in the literature have been in this disease and were 23.4 gm. by Schumacher, Williams, and Coltrin,[25] and 18.4 gm. by Foord and Randall.[6] Of the 13 cases of hyperproteinemia reported from the Boston City Hospital,[10] 7 had multiple myeloma. Although in the present study there were only 2 cases of this disease, neither of which showed hyperproteinemia, the author has since learned directly of 4 cases which were detected because of the hyperproteinemia found in their blood, analyzed for other reasons. From a review of the literature, Jeghers and Selesnick concluded that 50% to 60% of the cases of multiple myeloma have hyperproteinemia and that the serum protein determination is a more valuable aid in its diagnosis than the search for Bence-Jones protein in the urine. Ulrich[29] stated that hyperproteinemia is frequently present in the absence of Bence-Jones protein in the urine and *vice versa*. Since the tumor cell of multiple myeloma is the plasma cell, and since the concentration of serum globulin is so markedly and frequently increased in this disease, this suggests that the plasma cell is a source of formation of serum globulin. The plasma cell is considered by some to be a part of the reticulo-endothelial system.

Peters and Eisenman[22] found that metastasis to the bone marrow from primary carcinoma elsewhere may be associated with hyperproteinemia. Such cases usually have metastases to the liver as well. Metastases of carcinoma to the liver are frequently associated with hyperglobulinemia.[16] It is difficult, therefore, with the evidence at hand to determine whether the metastasis to the liver or that to the bone marrow is responsible for the hyperglobulinemia in these cases.

Chronic Infections. Of the 50 cases of hyperglobulinemia studied in this series, 31 were due to infection (Table 1). These infections were all chronic, either suppurative or non-suppurative. None was acute.

In 30 cases of acute infection, both suppurative and non-suppurative (not included in this table), such as pneumonia, mastoiditis, tonsillitis, cervical adenitis, etc., there was no increase in total serum protein above normal and only 2 showed slight increase in serum globulin.

The exact nature of the globulin increase in the chronic infections

is not known. If the entire increase of serum globulin were due to active antibodies, one might logically expect that the body would thereby be protected against such prolonged and serious infections. Perhaps much of this serum globulin is not active antibody but is rather the result of antibody antigen interaction.[23] Perhaps the increase is a compensatory mechanism for the absence of some missing factor such as complement.

TABLE 1.—SUMMARY OF DATA ON HYPERPROTEINEMIA AND HYPERGLOBULINEMIA

	Total protein				Globulin	
	No. cases	No. deter.	No. cases > 7.5 gm. per 100 cc.	No. deter. > 7.5 gm. per 100 cc.	No. deter.	No. deter. > 3 gm. per 100 cc.
Dehydration:						
Various causes	22	28	17	17	20	15
Diseases involving bone marrow:						
Monocytic leukemia	1	4	1	4	4	4
Multiple myeloma	2	4	0	0	4	1
Lymphatic leukemia	1	1	1	1	1	1
Infections:						
Abscess of lung	4	6	4	5	2	2
Bronchiectasis	5	7	4	4	1	1
Chronic pyonephrosis	1	4	1	1	2	2
Chronic osteomyelitis	1	1	0	0	1	1
Boeck's sarcoid	1	1	0	0	1	1
Tuberculosis	12	29	3	4	20	16
Syphilis	12	15	6	6	5	3
Venereal lymphogranuloma	11	11	8	10	8	7
Rheumatoid arthritis	1	1	1	1	1	1
Bacterial endocarditis	7	14	2	6	3	2
Lupus erythematosis disseminatus	2	3	2	2	2	2
Periarteritis nodosa	2	4	0	0	4	4

Of the 9 cases of pulmonary non-tuberculous suppuration (lung abscess and bronchiectasis) all but 1 had total protein concentrations above 7.5 gm. at some time in their course. The highest values seen in any of the infections were in patients with pulmonary abscess. These cases like those having chronic pyonephrosis and chronic osteomyelitis may be considered in a group of chronic suppurative infections in which hyperproteinemia is apparently common.

Hyperglobulinemia was also found in the chronic non-suppurative infections: tuberculosis, Boeck's sarcoid, syphilis, venereal lymphogranuloma, rheumatoid arthritis, bacterial endocarditis, lupus erythematosis disseminata, and periarteritis nodosa. The literature indicates that leprosy and kala-azar should also be added to this group.

In tuberculosis, Boeck's sarcoid, syphilis, venereal lymphogranuloma, leprosy, and kala-azar the mononuclear cell and macrophage are a prominent part of the pathologic picture. Furthermore, the bone marrow in the latter 5 diseases is said to resemble that in multiple myeloma because of the accumulation of plasma cells. The presence of hyperglobulinemia in these conditions emphasizes again, therefore, the relationship between the reticulo-endothelial system and globulin formation.

In an article by Eichelberger and McCluskey[5] there are tables showing results of 150 determinations of plasma proteins on their

109 patients with tuberculosis. Study of these tables reveals that the total protein concentration was above 8 gm. in 44% of the determinations, the globulin concentration above 3 gm. in 62% and the albumin below 4 gm. in 17%. In none of their cases was the total protein concentration below normal. These changes were essentially the same as those found in 12 cases of tuberculosis studied in the present series and in 38 cases studied by Stempien and Kagan[26] at the Willard Parker Hospital. In the latter 50 cases, however, hypoalbuminemia and decreased total protein were seen with greater frequency. In tuberculosis the infection probably is responsible for the increased serum globulin concentration and malnutrition is probably responsible, for the most part, for producing the decreased albumin concentration.

Kampmeier, Smith and Larsen[18] found hyperproteinemia in 62 of the 67 cases of venereal lymphogranuloma which they studied and considered this fact to be of diagnostic value. Hyperproteinemia was present in 8 of our 11 cases.

In our only case of rheumatoid arthritis, hyperproteinemia was present. Davis[4] found this to be most marked in the severe cases and noted normal serum protein in the quiescent stages. He considered the increased globulin to be further evidence of the infectious nature of this disease. Taussig[28] found similar changes in the blood of 2 children with Still's disease.

Kürten[19] found hyperproteinemia in 33 of his 35 cases of bacterial endocarditis. It was present in 5 of the 7 cases in this series at some time in their course. Two cases of lupus erythematosis and 2 cases of periarteritis nodosa were also associated with increased globulin. The latter three generally fatal diseases have a number of other clinical similarities, and pathologically they are often considered together.

Hyperglobulinemia was seen in 50% of the determinations done in a study of patients with acute glomerulonephritis.[17] The increase in globulin in this condition is probably again due to the associated infection. It is to be noted that this is one of the three conditions mentioned above in which the serum albumin concentration is sufficiently lowered so that the total protein concentration is often normal in spite of the globulin increase.

Of the infections studied, only abscess of the lung, subacute bacterial endocarditis, and venereal lymphogranuloma were associated with total protein concentrations above 9 gm.

Dehydration. The 22 cases of dehydration were secondary to various causes such as alcoholism, diarrhea, and coma. Of the 28 determinations, 17 showed an increase in total protein. In each of these cases in which globulin was determined it was the responsible factor. In none of the cases was the albumin concentration increased, and in 4 it was below normal. Three of the latter had had chronic diarrhea, which accounted satisfactorily for the hypoalbuminemia in spite of dehydration. The 4th patient was a chronic alcoholic who had long been on a protein starvation diet.

One would expect that in dehydration serum albumin would become

concentrated just as are the other blood constituents such as globulin and hemoglobin. However, we have not found this to be the case in any of the 22 cases of dehydration studied, even though most of these showed increased globulin and hemoglobin concentrations. This finding has been borne out in other patients whom we have since studied during the progress of dehydration. Just what happens to the albumin which is lost from the blood stream during the process of dehydration is not apparent. Talbott,[27] in 1935, felt that an increase in capillary permeability during dehydration might account for loss of some of the albumin from the blood. The observation of a slight albuminuria in some patients during marked dehydration would seem to be familiar evidence to support this. One may question, however, whether the amount which is lost in this manner, even when all the capillaries throughout the body are involved, is sufficient to account for the large amount of albumin lost from the blood in some cases of dehydration. Both Kürten[20] and Bing[1] have found that when serum is heated for ½ hour at 57° C. some of the albumin is converted to globulin. Whether or not this might occur within the body under any circumstances has not been reported. We are, therefore, faced with these questions: Is there an upper threshold of serum albumin concentration? If there is, how constant is it and by what mechanism is it maintained?* The answers to these questions are obviously of great clinical importance.

Mandelbaum,[21] in 1936, reviewed the various causes of dehydration and discussed the associated hyperproteinemia. Bridge and Cohen[3] reported on the value of serum protein concentration as a guide in the treatment of dehydration.

Shock. In this study there was no opportunity to study the increases in total protein concentration in shock which have been reported by a number of investigators.

Summary. The significance of hyperglobulinemia has been discussed from a clinical and physiologic point of view. Hyperglobulinemia appears principally in diseases involving the bone marrow, chronic infections, diseases involving the liver, and in dehydration.

The accompanying table classifies the cases studied in accordance with the above discussion. According to this study and the literature, values for total serum protein concentration over 9 gm. are rarely found except in multiple myeloma, monocytic leukemia, venereal lymphogranuloma, pulmonary suppuration, Boeck's sarcoid, subacute bacterial endocarditis, and dehydration. (A few diseases which are rare in this country, such as kala-azar and schistosomiasis, may also produce such changes.)

The presence of an upper threshold of albumin concentration in dehydration is suggested. Increase in the total protein concentration during dehydration in the cases studied was due to an increase in serum globulin while the serum albumin remained at the upper level of normal or was decreased due to other factors.

* Experiments to help determine these points were begun, but because of the author's entering the Armed Services were not carried to completion.

The clinical data presented support the theory that serum globulin is formed by the plasma cells and the reticulo-endothelial system throughout the body.

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CALCIUM SALTS OF SULFADIAZINE AND SULFATHIAZOLE: WITH PARTICULAR REFERENCE TO THEIR SUBCUTANEOUS ADMINISTRATION*

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Since the original report of Taplin and his associates[18] concerning the administration of sodium sulfapyridine by hypodermoclysis, the sodium salts of sulfapyridine, sulfathiazole, and sulfadiazine have been used extensively in this manner at the University of Minnesota Hospi-

* Aided by a grant from the Graduate School, University of Minnesota Medical School.

† On active service with the Armed Forces.

tals. For subcutaneous infusions, concentrations varying from 0.3% to 1% in sterile physiologic sodium chloride solution are used. No tissue irritation has resulted from this procedure. It was observed that if aqueous solutions of sodium sulfapyridine were used in concentrations exceeding 1%, an inflammatory reaction, and sometimes necrosis, might be provoked. Similar results followed the use of aqueous solutions of sodium sulfathiazole, particularly when concentrations of 3% to 5% were employed. Because of these observations, we have been reluctant to utilize solutions of sodium sulfadiazine for subcutaneous infusions in concentrations above 1%.

The administration by hypodermoclysis of solutions of the sodium sulfonamide compounds is advantageous in the treatment of certain groups of patients. On the medical services, patients having severe infections are frequently encountered who are unable to ingest the sulfonamides because of coma, delirium or vomiting. In these instances patients are given the elected sodium compound intravenously to attain adequate blood concentrations, and then the levels are maintained by subcutaneous infusion until the individuals are able to tolerate the drug by mouth. This procedure is also followed on the surgical services in the treatment of patients with postoperative infections. A further advantage of such a therapeutic approach is that the fluid and electrolyte balance is sustained. At times, it becomes increasingly difficult to give repeated intravenous injections of the sodium sulfonamide compounds to small children, and the subcutaneous route has been used successfully.

During recent months, sulfadiazine has been used extensively at the University Hospitals and elsewhere, particularly for the treatment of streptococcal and pneumonococcal infections. A number of reports have appeared in the literature, discussing the absorption, excretion, diffusion, toxicity and therapeutic effectiveness of sulfadiazine and its sodium salt in man. [1,4–8,13–16,19,20] Ordinarily, sodium sulfadiazine is given intravenously as a 5% solution in distilled water. A 5% concentration of sodium sulfadiazine is strongly alkaline, having a pH value around 9.5 which would appear to preclude its use by the subcutaneous route. For subcutaneous administration, the drug is usually given in 0.3% to 1% solutions in physiologic saline. Jorgenson and Greeley[11] have given to infants and children a 5% aqueous solution of sodium sulfadiazine by the subcutaneous route, and report that no tissue irritation followed such a procedure. The experimental studies of Fox[9] would indicate that sodium sulfadiazine is less irritating to the tissues of animals than the other commonly used sodium sulfonamide salts. More recently, we have treated a few adult patients with aqueous solutions of sodium sulfadiazine given subcutaneously in concentrations varying from 1% to 5%. Local tissue irritation and tenderness was observed with concentrations of 3% to 5%. Until more favorable evidence is available, the routine use of sodium sulfadiazine for subcutaneous infusions shall not exceed a concentration of 2%. The possibility of using a sulfadiazine preparation which would permit a more concentrated solution for subcutaneous administration prompted

us to evaluate a calcium salt of sulfadiazine, and also of calcium sulfathiazole.*

Materials and Methods. Calcium sulfadiazine is a white, crystalline, odorless and slightly bitter compound. It is less soluble than sodium sulfadiazine, having a solubility of about 5.1% in distilled water at 25° C. The compound tends to precipitate out in this concentration when physiologic saline solution is used. Calcium sulfadiazine solutions are less alkaline than the sodium salt. The pH values for different concentrations of this compound in aqueous solution as determined with the glass electrode are as follows:

5% calcium sulfadiazine in distilled water—pH 8.5
4% " " " " " —pH 8.4
3% " " " " " —pH 8.4
2% " " " " " —pH 8.3
1% " " " " " —pH 8.1

Calcium sulfadiazine was administered parenterally to a total of 24 adult patients. These men, convalescing from mild illnesses of various sorts, ranged in weight from 56 to 83 kg. All had normal renal and cardiac function. They were maintained on a general diet with a fluid intake of 2000 to 2500 cc. daily. For the injections, 4% solutions of calcium sulfadiazine in sterile distilled water were used. The pH of these solutions was 8.4. An initial dose of 4 gm. of drug was given. In 5 subjects the first injection was given subcutaneously while in 5 others the "loading" dose was administered intravenously. In all instances the first dose was followed at 12 hour intervals by two additional subcutaneous doses of 3 gm. each. Approximately 10 minutes were taken for the subcutaneous injections and 20 minutes for the intravenous injections. No generalized reactions resulted from the administration of the drug by these methods. Local reactions, except for slight tenderness which occasionally occurred at the site of subcutaneous injection, were also notably absent. Blood samples for the determination of drug levels were taken at frequent intervals for at least 36 hours. Quantitative determinations of the drug in the blood were made by the method of Bratton and Marshall.[2]†

The value of the subcutaneous administration of calcium sulfadiazine in establishing and maintaining effective drug levels in the blood was then observed in 14 patients suffering from a variety of infections. The majority of these patients had pneumonia. In each instance, the patient received an initial dose of 4 gm. of calcium sulfadiazine in a 4% aqueous solution subcutaneously. This was followed by two more subcutaneous doses of 3 gm. each in a 4% aqueous solution, given at 12 hour intervals. Thereafter, each patient received sulfadiazine by mouth in doses of 1 gm. every 4 or 6 hours, as needed, to maintain a total drug level of more than 7 mg. per 100 cc. of blood.

Blood Levels Following the Parenteral Administration of Calcium Sulfadiazine. The levels of free sulfadiazine in the blood after the subcutaneous administration of 4 gm. of calcium sulfadiazine, followed by two subcutaneous injections of 3 gm. each at 12 hour intervals, are shown in Figure 1. Maximum blood levels were usually obtained 4 or 5 hours after the subcutaneous injections. Relatively high blood levels were maintained for a period of approximately 10 hours after each dose. These blood concentrations compare favorably with those observed by other investigators after the oral administration of sulfadiazine itself or the subcutaneous injection of its sodium salt.[13–15,20] In our series,

* Dr. George A. Harrop, Director of the Squibb Institute for Medical Research, had these salts prepared and placed at our disposal.

† The sulfadiazine blood levels were carried out under the supervision of Dr. G. Evans, Director of Laboratories, University Hospitals.

the rate of excretion of the drug, as measured by its disappearance from the blood, did not differ significantly from that reported by the above authors. When the drug was withdrawn, the blood level of total drug fell to less than 2 mg. per 100 cc. by the end of 48 hours after the last injection.

The sulfadiazine levels in the blood after an initial intravenous dose of 4 gm. of calcium sulfadiazine, followed by two subcutaneous injections of 3 gm. each at intervals of 12 hours, are shown in Figure 2. Immediately after intravenous administration, the drug concentration in the blood was high although the actual drug levels varied considerably in different subjects. Subsequently there was a steady decline of

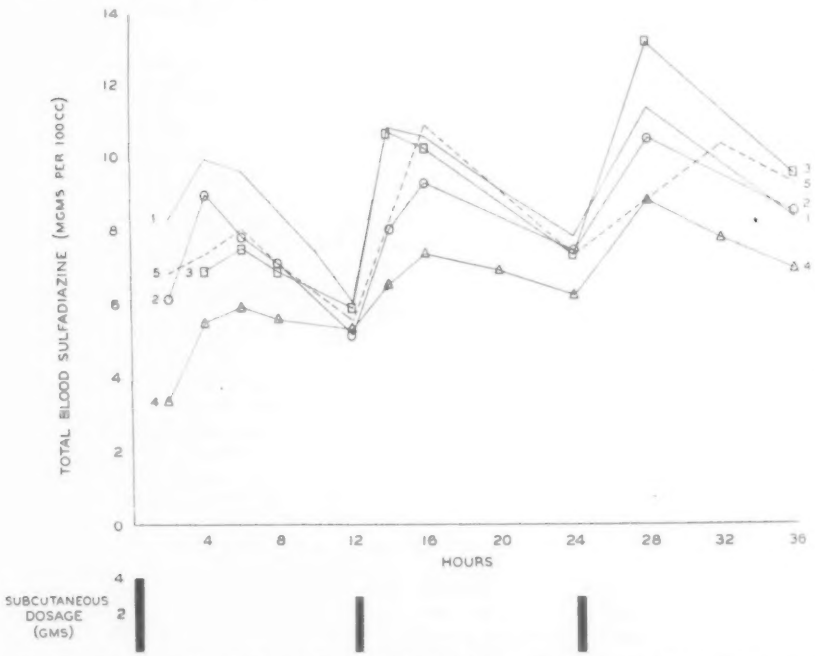

FIG. 1.—Concentrations of free sulfadiazine in the blood following repeated subcutaneous injections of calcium sulfadiazine.

the blood levels until the first subcutaneous dose was given. Nevertheless, it is clear that the intravenous injection of 4 gm. of calcium sulfadiazine, given as a 4% solution in distilled water, is an effective method for quickly establishing high levels of the drug in the blood. These blood levels may then be maintained and regulated by the judicious subcutaneous administration of different amounts of this compound.

Therapeutic Results. The essential data concerning 14 patients who were treated by the subcutaneous administration of calcium sulfadiazine for 24 hours, followed by sulfadiazine orally, are outlined in Table 1. These patients suffering from a variety of infections were

all acutely ill.* In each case the concentration of total sulfadiazine in the blood exceeded 7 mg. per 100 cc. within 3 hours after the first injection of drug. Thereafter the blood levels of sulfadiazine in these patients, during both parenteral and oral therapy, consistently tended to exceed the levels observed in control subjects after similar dosage. Domm and his associates[4] have suggested that this may be the result of impaired renal function during the height of illness. For the most part, total drug levels in the blood of these patients during treatment ranged from 7 to 15 mg. per 100 cc. In some cases, however, levels up to 20 mg. per 100 cc. were occasionally reached. Unless otherwise

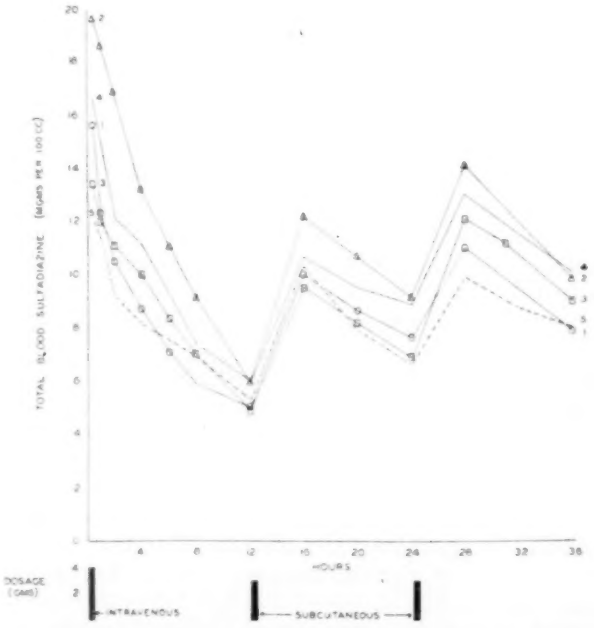

FIG. 2.—Concentrations of free sulfadiazine in the blood after an initial intravenous injection of calcium sulfadiazine, followed by two subcutaneous injections of the calcium salt.

noted, chemotherapy was continued for at least 48 hours after the patient's temperature had returned to normal or after unmistakable clinical signs of improvement had appeared. No severe toxic reactions attributable to the drug were observed in this series of patients, and there was an absence of nausea and vomiting. The skin eruption seen in 1 patient was confined to the face and upper extremities. It was macular in character and subsided promptly when the drug was discontinued. The microscopic hematuria exhibited by 2 patients was not sufficiently severe to warrant withdrawal of the drug.

* Bacteriologic data was obtained under Dr. Milton Levine, Director of the Bacteriology Laboratories, University Hospital.

Calcium Sulfathiazole. Only a few observations have been carried out with this compound in human subjects. Calcium sulfathiazole is a white, crystalline, odorless and slightly bitter compound. It is slightly less soluble than calcium sulfadiazine, but 10 times less alkaline than sodium sulfathiazole. Varying concentrations of solutions of calcium sulfathiazole are slightly more alkaline than comparable solutions of calcium sulfadiazine. The pH of a 5% aqueous solution of calcium sulfathiazole is 8.8.

The behavior of this compound following parenteral administration was observed in 5 adult males. These men ranged in weight from 53 to 73 kg. and were otherwise clinically comparable to the group which received trial injections of calcium sulfadiazine. Each injection was prepared from 4% solutions of calcium sulfathiazole dissolved in sterile distilled water at 80° C. and then cooled to 37° C. Each subject received an initial dose of 4 gm. intravenously followed in 12 hours by a subcutaneous dose of 3 gm. In 3 cases, further subcutaneous administration of sulfathiazole was discontinued because of redness, induration, and pain at the site of injection. Two patients received a second subcutaneous injection of 3 gm. of the drug 12 hours after the first injection with no untoward local symptoms. No generalized reactions resulted from the administration of calcium sulfathiazole by these methods. The sulfathiazole concentrations in the blood were one-half to two-thirds that obtained with calcium sulfadiazine under similar conditions.

Comment. The data presented here indicate that the calcium salt of sulfadiazine can be used safely and effectively in patients requiring parenteral chemotherapy. Solutions of this drug in distilled water are not strongly alkaline and have been administered subcutaneously as well as intravenously with no ill-effects in concentrations up to 4%. This allows considerable latitude in the regulation of dosage and in the maintenance of the desired blood levels. The rate of disappearance of drug from the blood after the injection of calcium sulfadiazine is not significantly different from that observed following the parenteral administration of comparable doses of sodium sulfadiazine. Thus, blood levels of a desired magnitude can be easily maintained by injections of calcium sulfadiazine at intervals of as much as 12 hours. The amount of drug and amount of fluid administered with each dose may be varied according to the needs of the individual patient.

It has long been known that the injection of ionized calcium salts may cause characteristic exudative lesions which provide peculiarly favorable cultural conditions for the growth of streptococci and anaërobic bacilli.[3,12] This phenomenon, called *kataphylaxis* by Bullock and Cramer,[3] apparently is not due to any increased virulence of the bacteria but to a reduction of local tissue resistance. In our series of patients, there were no instances of severe tissue reactions or superimposed infections at the site of injection of calcium sulfadiazine.

It has been reported that the intravenous injection of calcium salts into fully digitalized patients has precipitated sudden death.[10] How-

ever, Smith and his associates were not able to confirm this phenomenon experimentally.[17] Calcium sulfadiazine has been administered intravenously to fully digitalized individuals without any ill-effects.

TABLE 1.—SUMMARY OF DATA FOR 14 PATIENTS WITH MISCELLANEOUS INFECTIONS TREATED WITH CALCIUM SULFADIAZINE ADMINISTERED SUBCUTANEOUSLY AND SULFADIAZINE GIVEN BY MOUTH

Case No.	Age (yrs.)	Sex	Treatment begun, day of disease	Diagnosis	Total dose of calcium sulfadiazine and sulfadiazine (gm.)	Toxicity	Response	Comment
1	33	M	17	Infectious arthritis	28	....	Good	M. tetragenus isolated from joint fluid
2	81	F	3	Bronchopneumonia	35	Erythematous skin eruption 7th day	Good	
3	68	M	2	Bacteremia (beta hemolytic streptococcus)	29	....	Good	
4	68	M	5	Lobar pneumonia (pn. Type I)	21	Crystalluria occasional red blood cells in urinary sediment	Death 4th day	Leukopenia on admission, initial blood culture positive (4900 organisms per cc.), subsequently sterile; 150,000 U. pn. Type I antiserum given; Francis test positive 2d day; autopsy: lobar pneumonia all lobes with empyema, right
5	64	F	1	Lobar pneumonia (pn. Type III)	22	....	Good	Left lower lobe involved; blood cultures sterile
6	60	F	3	Lobar pneumonia (pn. Type I)	25	....	Good	Left lower lobe involved; blood cultures sterile; mild right heart failure
7	62	M	4	Bronchopneumonia	19	Crystalluria, occasional red blood cells in urinary sediment	Good	
8	43	F	1	Bronchopneumonia	10	....	Death 25 hours	Thyroidectomy 2 days postoperative; pn. Type XXIII recovered from sputum; chronic alcoholism; autopsy: bronchopneumonia right lower lobe; left heart failure
9	58	F	2	Lobar pneumonia	27	....	Fair	Blood culture sterile; no pneumococci typed
10	35	M	47	?Blastomycosis; ?generalized coccidioidomycosis	26	Marked crystalluria	Death 16th day	Chemotherapy discontinued on 5th day; pulmonary involvement of all lobes; diffuse enteritis; no autopsy
11	40	M	4	Lobar pneumonia (pn. Type I)	66	....	Excellent	Initial blood culture, 5000 pn. Type I per cc.; subsequent cultures sterile; 100,000 U type specific antipneumococcus serum given; Francis test positive 2d day; entire left lung involved; sterile pleural effusion
12	46	M	10	Lobar pneumonia (pn. Type II)	22	....	Fair	Left lower lung involved; sterile pleural effusion; blood culture sterile
13	26	M	9	Lobar pneumonia (pn. Type I)	34	....	Fair	Empyema (pn. Type I) on right; surgical drainage; recovery uneventful
14	34	M	1	Lobar pneumonia (pn. Type XXVII)	29	....	Good	Left lower lobe involved; blood culture sterile

Preliminary investigations with the calcium salt of sulfathiazole indicate that its use may be accompanied by inflammation and necrosis, particularly in concentrations comparable to those used for calcium sulfadiazine. We do not recommend its clinical use. Data at hand do not permit us to draw any conclusions concerning the therapeutic efficiency of calcium sulfathiazole.

Summary. 1. Aqueous solutions of calcium sulfadiazine may be administered subcutaneously or intravenously in concentrations up to 4% with no ill-effects.

2. The pattern of absorption and excretion of calcium sulfadiazine, as measured by the rise and fall of drug level in the blood, does not differ significantly from that observed following the parenteral administration of comparable doses of sodium sulfadiazine.

3. Preliminary clinical experience indicates that calcium sulfadiazine administered subcutaneously is effective in establishing and maintaining adequate blood levels of drug in patients requiring parenteral chemotherapy.

4. Aqueous solutions of calcium sulfathiazole administered subcutaneously resulted in local inflammation.

5. It has been reported that aqueous solutions of sodium sulfadiazine in concentrations up to 5% may be safely administered subcutaneously. If these observations are confirmed, there appears to be no advantage in utilizing calcium sulfadiazine for this purpose.

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THE TREATMENT OF PNEUMOCOCCAL PNEUMONIA WITH SULFAPYRAZINE*

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This paper is an amplification of a preliminary report[4] from this laboratory of the use of sulfapyrazine†[1,3] in pneumococcal pneumonia. Sulfapyrazine's antipneumococcal activity in experimental infections in mice has been shown to be at least as great as that of sulfadiazine and sulfathiazole, and its *in vitro* effectiveness greater than that of sulfadiazine but less than that of sulfathiazole.[6] Its absorption, excretion, and distribution in man resemble, in general, those of its isomer sulfadiazine.[2] Oral and parenteral administration of the drug to mice, rats, dogs, and monkeys[4,5] has indicated that its toxicity is low.

General Plan of Study. All the patients in this series were treated on the wards of the Cincinnati General Hospital. The patients were seen daily by one or more members of the group during the course of treatment and, when possible, examined by one member before sulfapyrazine therapy was instituted. The only criterion employed in the selection of cases was the diagnosis of "typical" pneumonia as determined by history, and physical examination or roentgenography. A blood culture and sputum sample were collected from each patient before the institution of chemotherapy; in a few instances, when sputum was not obtainable, exudate from a throat swab was inoculated into culture media. Treatment was never delayed, however, until the causative organisms were identified. When a blood culture showed growth, daily cultures were taken until at least 2 "negative" cultures had been reported; culture media contained 5 mg. per 100 cc. para-aminobenzoic acid. During the period from March 1, 1941 to June 30, 1942, about 40% of the adult patients suffering from pneumonia were treated with sulfapyrazine, while the great majority of the remainder received sulfathiazole. Antipneumococcal serum was given to those patients who did not respond promptly to chemotherapy, or, sometimes, to especially ill patients admitted in the fourth or fifth day of pneumonia.

Dosage of Sulfapyrazine. During the early part of this study, all patients were given an initial dose of 2 or 4 gm. followed by 1 gm. every 4 hours; all the drug was given orally. Later, it became our practice to administer the initial dose of 4 gm. intravenously, as a 5% solution of the sodium (monohydrate) salt, and to continue the maintenance dosage as before. Because of the appearance of instances of renal irritation in a few patients, the mainte-

* This study was aided by a grant in honor of Craig Yeiser and by a grant from Mead Johnson & Company.

† The sulfapyrazine was furnished by Mead Johnson & Company.

nance dose was changed to 1 gm. every 6 hours; no patient receiving this dose showed signs of renal toxicity. The therapeutic efficacy of the drug appeared to be just as great with the smaller total dose. Sulfapyrazine was administered routinely until the rectal temperature remained below 99° F., for 48 hours; an average total dose of 30.4 gm. was given to the patients in this series.

Type Distribution. The sulfapyrazine-treated group comprised 105 cases, whose type distribution is shown in Table 1. Types I, II, III, V, VII and VIII accounted for more than 70% of the total; in 12 instances, no etiologic diagnosis could be made by blood or sputum culture. Twenty-four (23%) of the patients had bacteremia at the time chemotherapy was instituted.

TABLE 1.—DISTRIBUTION OF PNEUMOCOCCUS TYPES

Type	No. of patients	No. of deaths	No. of patients with bacteremia
I	22	0	8
II	9	0	3
III	8	0	2
IV	2	0	0
V	9	1	4
VI	1	0	0
VII	15	0	1
VIII	9	0	2
IX	1	0	0
XI	1	0	0
XII	1	0	0
XIII	1	0	0
XIV	3	1	1
XVI	2	1	1
XVIII	2	1	1
XIX	2	0	0
XXIV	1	0	1
XXVII	3	0	0
XXXIII	1	0	0
Untyped	12	0	0
Totals	105	4	24

TABLE 2.—MORTALITY IN PNEUMOCOCCAL PNEUMONIA, CINCINNATI GENERAL HOSPITAL MARCH 1941–JUNE 1942

	Drug	
	Sulfathiazole, July 1941–June 1942	Sulfapyrazine, March, 1941–June 1942
All cases	133	105
Number of deaths	9	4
Mortality, %	7	4
Non-bacteremic cases	107	69
Number of deaths	4	0
Mortality, %	4	0
Bacteremic cases	26	24
Number of deaths	5	4
Mortality, %	19	17

Results of Treatment. (See Table 1.) All of the patients, except 4, recovered. The 4 fatal cases occurred in patients with bacteremia. Two of these patients received serum in fairly large doses in addition to the sulfapyrazine, while a third received human plasma. With one exception, the fatalities occurred in patients over 50 who gave evidence of degenerative disease. For purposes of comparison, the mortality figures for sulfathiazole-treated pneumonia at the Cincinnati General Hospital during the same period are shown in Table 2; none of the patients

receiving sulfathiazole received serum. It can thus be seen that there was no striking difference in the curative value of the two drugs.

Even though sulfapyrazine is in no sense an antitoxic substance, it seemed to share the property of certain of the other sulfonamides of diminishing the signs of "clinical toxicity" so frequently associated with pneumococcus pneumonia. This improvement in clinical condition was usually apparent in 12 to 24 hours and was apparently independent of the patient's temperature. A critical fall of temperature occurring after the administration of the drug was noted in 45% of the non-bacteremic cases and in only 16% of the recovered bacteremic patients; similar incidences might be expected in untreated patients. The details of the fatal cases are appended below.

The celerity with which blood cultures from bacteremic patients may become "negative" under sulfonamide treatment is striking. This property of sulfapyrazine was exemplified in 2 patients with 350 and 680 colonies per cc., respectively, of Type V pneumococci; within 24 hours the blood culture showed growth only in the flasks and on subsequent examinations they were sterile. In another case, the blood culture showed countless colonies of Type XVIII pneumococci before treatment, but was sterile 16 hours after an intravenous injection of 4 gm. of sodium sulfapyrazine. No patient in this series developed a purulent complication of pneumonia under treatment. However, 2 patients were admitted with focal purulent complications: 1 with infected pleural fluid containing pneumococcus I, and another with pyarthrosis of the knee and sternoclavicular joints due to pneumococcus V. The pyarthroses disappeared completely without requiring surgical drainage, but the empyema ultimately required rib resection.

Drug Toxicity. There was no proved instance of sulfapyrazine causing a deleterious effect upon the central or peripheral nervous system. In 3 cases, delirium occurring during treatment was thought by some observers to be due to the drug, but readministration of the drug, in similar dosage, did not cause a repetition of the delirium in these patients. There was 1 instance of nausea and vomiting and 2 instances of fever which may have been due to the drug. One patient developed a morbilliform eruption during the course of treatment, but the rash could not be duplicated during convalescence, when sulfapyrazine treatment was reinstituted. No cyanosis or jaundice associated with chemotherapy was seen.

The major toxic effects of sulfonamides on the red and white blood cells were not encountered among patients of this series. In 1 instance there was a decrease in the white blood cell count from 10,900 to 2700, with 43% polymorphonuclears, observed. The drug was readministered during convalescence after the total count had returned to normal but no effect was noted, either quantitatively or qualitatively.

In 5 of 57 patients given 6 gm. of sulfapyrazine a day, an elevation of the blood urea nitrogen, associated with pain in the flank, hematuria, and oliguria, occurred during treatment; in no instance did anuria supervene. In 2 cases, the blood urea nitrogen rose from 18 mg. per 100 cc. and 21 mg. per 100 cc., respectively, on admission, to 49 mg. per 100 cc. and 61 mg. per 100 cc., and in the third case from 42 mg.

per 100 cc. to 90 mg. per 100 cc. In 2 other cases, where determinations of the blood urea nitrogen were not made on admission, elevations to 50 mg. per 100 cc. and 143 mg. per 100 cc. were discovered later. No patient developed any clinical signs of uremia, and in every instance the blood urea nitrogen returned to normal following discontinuance of the drug and proper attention to fluid intake. Among 44 patients treated with 4 gm. a day, no significant signs of renal toxicity were seen, though a few red blood cells were discovered microscopically in 6 cases.

Summary of Fatal Cases. Case 1. This 31 year old negress worked until the day before admission to the hospital. She gave a typical history of pneumococcal pneumonia of 24 hours' duration. On admission she was acutely ill with a temperature of 105.8° F., pulse 130, respirations 44, and blood pressure 130 systolic and 70 diastolic. There were classical signs of consolidation of the left upper lobe, but the lungs were otherwise clear. The white blood cell count was 5000 with 80% neutrophils. The red blood cell count was 3,700,000, hemoglobin 12.5 gm. The urine was normal. The blood urea nitrogen was 21 mg. per 100 cc.

She was given 4 gm. of sodium sulfapyrazine intravenously 1½ hours after admission. Six hours later the blood pressure had fallen to 70 systolic, and moist râles were present throughout the lungs. She died a few minutes later, despite efforts at resuscitation. Her total dose of sulfapyrazine was 5 gm. Type V pneumococci were later recovered from a culture of a throat swab, and from the broth of the admission blood culture, though no colonies appeared on the agar plates. Permission for necropsy was refused.

Case 2. A 68 year old white woman, afflicted with asthma and chronic bronchitis for the previous 8 years, was admitted to the hospital with pneumonia of 7 days' duration involving the entire right lung. She was cyanotic and covered with sweat. The extremities were ice-cold. The temperature was 101.6° F., the pulse 120, respirations 28, and the blood pressure 80 systolic and 60 diastolic. Moist râles and rhonchi were heard over the entire chest. The white blood count was 14,400, and the urine contained albumin. The admission blood culture was positive for Type XIV pneumococcus.

She was given 5 gm. of sodium sulfapyrazine intravenously, and supportive treatment which included oxygen, intravenous plasma, and hourly intravenous injections of paredrine. Despite these measures, the pulse became weaker and weaker, and the systolic blood pressure dropped to 40 mm. Hg. She died 17 hours after admission.

Autopsy revealed gray hepatization involving the right upper and middle lobes, and patchy involvement of the right lower lobe, and slight edema of the left lung.

Case 3. A 55 year old white man was admitted in the 5th day of pneumonia of the right middle and lower lobes, due to Type XVI pneumococcus. He was a chronic alcoholic who had lost 55 pounds in the preceding year and a half, and had signs of cardiac decompensation. On admission the temperature was 101.8° F., pulse 132, respirations 28, and blood pressure 110 systolic and 70 diastolic. He was somewhat irrational. The skin was icteric, and the liver was enlarged and nodular. The white blood cell count was 3000, with 74% young neutrophils. Bile and albumin were found in the urine. The blood urea nitrogen was 23 mg. per 100 cc. The blood culture contained 376 colonies of Type XVI pneumococci per cc.

Four gm. of sulfapyrazine were administered by mouth, and 5 gm. more during the next 12 hours. Twelve hours after admission he was given 200,000 units antipneumococcal rabbit serum, but he grew steadily worse, and died 2 hours later. Permission for necropsy was refused.

Case 4. A 75 year old negro was admitted to the hospital on the 7th day of pneumococcus XVIII pneumonia of the left upper lobe. For 5 months previously he had suffered from cardiac decompensation. On admission his

temperature was 103° F., pulse 112, respirations 42, and blood pressure 75 systolic and 50 diastolic. The pupils were irregular and reacted poorly to light, the heart was enlarged, and coarse râles were present throughout both lung fields. The white blood cells numbered 28,500; the specific gravity of the urine was 1.024, and a few white blood cells were seen in the sediment. The blood urea nitrogen was 85 mg. per 100 cc. The admission blood culture contained countless colonies of Type XVIII pneumococci.

Four gm. of sodium sulfapyrazine were given on admission, and 200,000 units of antipneumococcal rabbit serum 16 hours later. A blood culture taken just before administration of the serum was sterile. Two more grams of sulfapyrazine were given by mouth, and supportive therapy administered in the form of oxygen, aminophyllin, and the plasma from 1 liter of whole blood. Nevertheless, the systolic blood pressure failed to rise above 100, and the patient died 30 hours after admission.

Autopsy revealed gray hepatization of the left upper lobe, severe toxic changes in the heart and kidneys, fatty infiltration of the liver, and fusiform syphilitic aneurysm of the ascending aorta.

Summary. The results of the use of sulfapyrazine in the treatment of 105 cases of "typical" pneumonia are presented. The drug bears out its original promise of being an effective agent in the treatment of pneumococcal pneumonia, for the mortality in this series was only 4%. The mortality among the 24 bacteremic cases was 17%.

The only important toxic effect of the drug was upon the kidneys. Evidence of transient renal damage was found in 9% of the patients treated with 1 gm. every 4 hours. In patients treated with 1 gm. every 6 hours, the only manifestation of renal injury was the occasional microscopic finding of small numbers of red blood cells. The incidence of renal irritation of sulfapyrazine in larger series of cases should be investigated.

The other toxic effects commonly produced by sulfonamide drugs appeared but rarely in this series. One morbilliform rash and 1 instance of nausea and vomiting were believed to be due to sulfapyrazine.

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STATUS OF SULFONAMIDE THERAPY IN MALARIA

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With the onset of the war in the Pacific it became evident that the supply of quinine and its derivatives to the Allied Nations would, to

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say the least, become drastically curtailed. Subsequently, and even prior to December 7, 1941, physicians and other interested investigators began to consider departures in anti-malarial therapy radically different from the tried and-accepted quinine salts. It had long been accepted that there was no ideal anti-malarial drug, quinine most nearly approaching the ideal and being sufficiently satisfactory to cool the ardor of those who would have sought further. Out of the circumstances culminating in the loss of the East Indies and the concomitant loss of 90% of the world's supply of quinine came further impetus for investigators in their search for the ideal and, indeed, any effective and readily available anti-malarial drug.

The problems of oiling and draining bodies of stagnant water, which serve as breeding places, while capable of solution in certain malarious areas, loom as utterly impossible of solution in others. It is therefore obvious that complete destruction of the Anophelini may never be accomplished. Since it is impossible to remove the vector from the cycle, the next most desirable procedure would appear to be the eradication of human reservoirs of infestation. Theoretically, the simultaneous screening of all infested individuals until biologic cure is attained would needs be considered as one method for accomplishing this end. Practically, this consideration would be discarded at once. It would be about as easy as eradicating syphilis by segregating all syphilitics and treating them simultaneously until all were arrested or cured. The remaining alternative would be the employment of one or more drugs, which would be considerably more effective than the venerable but epidemiologically, at least partially, ineffective quinine derivatives. In order to improve upon the malaria situation as we now find it, the new drug or drugs should be relatively inexpensive, easily accessible, not too toxic, and capable of ridding the blood stream of parasites within a relatively short time. To attain the maximum results, even with such a drug available, diagnostic facilities and the means of distribution and administration of the drug would also require further development. Withal, the new treatment should not of necessity require a long enough treatment period to try the patience of the individuals taking it. The long treatment period required with the use of quinine and the resulting desertion of the treatment schedule by patients has been one of the chief objections to the use of this drug. However, since December 7, 1941, any discussion of the defects of quinine would appear to be academic.

Sulfathiazole in Therapeutic Vivax and Malariæ Malaria. In January of 1942, the author treated a case of therapeutic quartan malaria, a neurosyphilitic, with sulfathiazole and independently found that a definite anti-malarial quality was possessed by the drug. This case, No. 13027, had had several paroxysms over 103° F., rectal. Between January 6 and 9, 1942, he was given 2 gm. of sulfathiazole q.i.d. (q. 4 hr.) for 24 hours followed by 1 gm. q.i.d. There were paroxysms on January 8 and 11. These were followed by two slight reactions of less than 102° F., and then a paroxysm with 104° F. on January 25. He was completely free of even low grade fever from January 13 to 19.

On January 25, the regular quartan pattern was resumed and was subsequently terminated with quinine sulfate. On February 14, 1942, Case 13065, who also had therapeutic quartan malaria, was begun on sulfathiazole, 1.5 gm. q.i.d. (q. 4 hr.), for 2 days and followed by 1 gm. t.i.d. for 2 days. From the time of the beginning of the sulfathiazole schedule there were no further febrile reactions and after discontinuation of the medication the patient remained fever-free until February 26, 1942, on which day there was a temperature elevation to 101.5° F. On March 1, there was a temperature of 102.6° F. Quinine therapy was begun on March 2, 1942. The next day there was a paroxysm with elevation to 104.2° F. There were no further febrile reactions. From observations in these 2 cases it was concluded that, while sulfathiazole demonstrated some anti-malarial action although failing to permanently interrupt the malaria course in either case, other members of the sulfonamide series should be investigated. Accordingly, a study of the effects of sulfadiazine in therapeutic quartan malaria was undertaken. Sulfadiazine was selected on account of its extremely low toxicity and its broad margin of safety.

In November, 1941, Schwartz *et al.*[5] published results of their work with sulfathiazole as an anti-malarial in 9 neurosyphilitics undergoing therapeutic tertian malaria. The patients were allowed to have from 3 to 15 chills before sulfonamide medication was begun. Three gm. was the initial dose and this was repeated in 4 hours. Thereafter 1. gm. was given every 4 hours for a total dosage of 25 to 50 gm. Chills and fever ceased in every patient after sulfathiazole but the blood was cleared of plasmodia in only 5 of the 9 cases. One of these suffered a relapse 15 days after discontinuation of sulfathiazole therapy. After a period of 90 days there had been no sign of relapse in the other 4. The remaining 4 patients, whose blood could not be cleared of plasmodia, all experienced clinical relapses in from 12 to 20 days after the chemotherapy was discontinued. There were no serious toxic manifestations in any of the 9 patients.

Work of Coggeshall and others. As early as 1938 sulfanilamide was employed by Coggeshall[1] in the treatment of *P. knowlesi* malaria in *Macacus rhesus* monkeys. In this type of malaria sulfanilamide effected complete sterilization of the blood of the test monkeys. His experience in this connection led him to the use of sulfanilamide in human malaria but here he found it without effect. On September 27, 1941, Coggeshall, Maier, and Best[2] published their results of the treatment of various types of malaria with promin (sodium P,P′-diaminodiphenylsulfone N,N′-didextrose sulfonate) and with sulfadiazine (2-sulfanilamido-pyrimidine). Seventeen cases were treated with promin, of which 12 were vivax and 5 falciparum infestations. In the vivax cases the time interval required for disappearance of fever varied from immediate to 6 days with an average of $2\frac{5}{6}$ days. Of these 12 cases, plasmodia persisted in the blood in 4 and required from 1 to 5 days for their disappearance in the remaining 8 cases with an average of $3\frac{3}{8}$ days. The treatment periods varied from 3 to 4 days in each case and the total dosage varied from 5 to 40 gm. In the falciparum cases, 5 in

number, there were none in which plasmodia persisted. Gametocytes persisted in 1 case for 10 days. In the remaining 4 all parasites disappeared in a period of 2 days. The duration of the attack in all 17 cases before treatment was begun varied from 1 to 14 days, averaging $5\frac{1}{17}$ days. Promin was then used in several patients who had therapeutic malaria but results here were inconclusive. One of these patients experienced a resumption of paroxysms. Four of them were treated with promin for 3 days after having had 10 paroxysms each. Blood smears became negative and fever disappeared within 2 days. There was no recurrence of symptoms or parasites for 6 days. The patients then received the routine course of quinine. From an experimental point of view the quinine should not have been administered until a considerably later period, if at all, in order to have determined

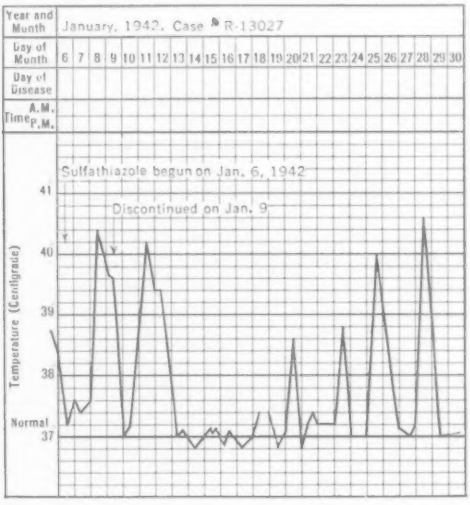
Year and Month
January, 1942. Case # R-13027
Day of Month
6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Day of Disease
Time A.M. P.M.
Sulfathiazole begun on Jan. 6, 1942
Discontinued on Jan. 9
Temperature (Centigrade)
41
40
39
38
Normal
37

FIG. 1.—Graphic representation of effect of 3 days treatment of Case 13027, a case of therapeutic quartan malaria. Sulfathiazole.

whether relapse would have occurred. If the patients were to have been released from observation within a short time the administration of quinine would have been proper since it had not been established that promin would cure therapeutic malaria and that paroxysms would not have resumed subsequent to release from observation.

These investigators also studied the effect of sulfadiazine on acute naturally acquired malaria. Thirteen cases were studied of which 7 were vivax infestations, 5 falciparum, and 1 malariæ. The number of days of duration of the attack before treatment had begun varied from 1 to 18 with an average of $4\frac{1}{3}\frac{2}{3}$ days. In 1 case of vivax and in 2 cases of falciparum infestation no effects were observed. In the remaining 10 cases fever disappeared in from $\frac{1}{2}$ to 3 days and plasmodia disappeared from the blood in from 1 to 5 days. The treatment period was the same in all cases and was for 6 days, 6 gm. being given on the

first day and 4 gm. daily for the remaining 5 days. From the fact that in 1 case 5 days were required for the parasites to disappear, it is possible that a treatment period of even more than 6 days may have been indicated.

The conclusion of these authors was that "at present there are no reasons for giving the drugs (sulfonamides) in preference to quinine or atabrine for the treatment of malaria, and they should be regarded only as important substitutes." In comment on this conclusion it should be pointed out that this paper was published before the East Indies were occupied by the Japanese and our supply of high grade quinine cut off. If the sulfonamides are to be regarded as important substitutes for quinine, it is apparent that now is the time to use them for that purpose.

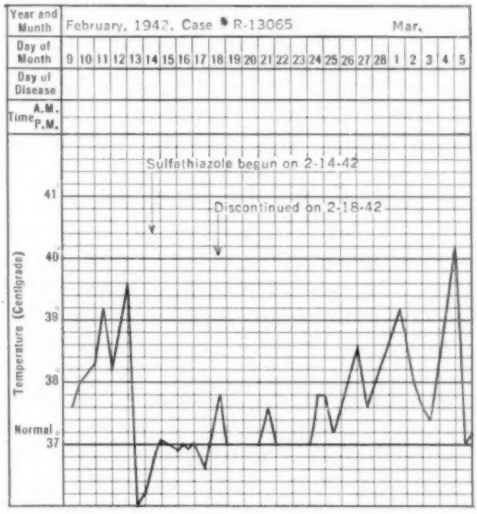

FIG. 2.—Effect of 4 days treatment of Case 13065, also a case of therapeutic quartan malaria. Sulfathiazole was also used here.

Relative Numerical Importance of Tertian and Quartan Therapy. The use of quartan malaria for therapeutic purposes has become much more general in the past few years, especially in the Pacific Coast area where it is used almost to the exclusion of tertian. Long range results of malaria therapy in neurosyphilitics are said to be slightly better with tertian than with quartan malaria but due to the milder course of the disease and to the smaller number of complications with corresponding lowering of mortality rates in the latter,[3] quartan therapy is becoming ever more popular with conservative neurosyphilologists. Results of the study of the effects of sulfadiazine in therapeutic quartan malaria (to follow) would therefore appear to be of greater import than would have been the case a decade ago.

Therapeutic tertian malaria, which has a marked tendency to spontaneous remission, requires a treatment period of only about 10 days

with relatively light quinine dosage. Obviously then, it does not constitute a problem of considerable dimensions. Moore,[4] who has had an enormous experience with therapeutic malaria, states that he has never seen a relapse in artificially induced tertian malaria following a proper quinine course. In contrast, therapeutic quartan malaria is characterized by a fairly high incidence of relapses and requires considerably more energetic treatment than does tertian.

The strain of quartan employed in our series has been in constant use for a period of more than 10 years and has long since become asexualized. It produces a very regular quartan fever pattern and spontaneous remissions never occur in it except in patients who have had natural or a previously induced course of therapeutic quartan malaria. It was considered ideal for a study of the effects of sulfadiazine on asexual artificially induced quartan malaria. It was also considered favorable that the patients under study could be kept under observation for a comparatively long time and that this would aid materially in determining the rate of relapse after treatment.

Sulfadiazine Therapy. Untoward Reactions. As stated above, sulfadiazine was selected for our work, from the sulfonamide field, largely on account of the low toxicity of the drug. Toxic reactions of any importance are apparently rare. One of the most common side effects, of a mild nature, is a state of mild confusion and cloudiness of sensorium which clears rapidly upon withdrawal of the drug and results in no permanent detrimental condition. Coggeshall *et al.*[2] comment on a case of malaria treated by them with sulfadiazine in which fever developed as a result of the medication and ceased at once upon its discontinuation. In our first series, of 10 malaria cases, treated with this drug there was 1 case of severe emesis which became active with each dose administered. Evacuation of the stomach occurred each time within a few minutes after the drug was taken. Even so, however, a sufficient amount of it was retained to clear the blood of plasmodia for a period of 39 days and to cause disappearance of febrile reactions. Although it is conceded that routine administration of sodium bicarbonate with sulfadiazine is not a preferred procedure, it has been our practice to give it when nausea developed during the course of medication. Accordingly, sodium bicarbonate was begun in this case but failed to check the vomiting. Finally the withdrawal of the sulfonamide became necessary in order to control it. In view of the emesis and of the consequent loss of a large proportion of the sulfadiazine, this case was not counted a relapse with sulfadiazine therapy.

No renal disturbances at all were noted in the above series. Wright and Kinsey[6] discuss cases of anuria resulting from the use of the drug. They state that it is their opinion that this will not occur if the dosage is kept at or under 4 gm. daily. In our work, with the exception of the first 24 to 48 hours during which larger doses were given for the purpose of building up blood levels, daily dosages have been 4 gm. or less. We are now following their suggestion of searching for the combined presence of sulfadiazine crystals and red blood cells in the urine specimens which we examine on all cases under sulfadiazine therapy. They

state that the presence of this combination indicates crystalline concretions in the ureters and constitutes an indication for discontinuation of the treatment schedule.

With the exception of the emesis case above described, no other toxic manifestations were observed in this series.

Technique of Administration. A set of standard conditions was established for the 10 cases constituting series No. 1. All patients were kept at bed rest but were allowed bathroom privileges. Otherwise they were kept continuously in bed and were served their meals there. No other medication was given during the sulfonamide course except sodium bicarbonate in the 1 case above discussed. The usual routine in our neurosyphilis clinic of 4 intravenous injections of mapharsen following the termination of malaria was abandoned on account of the anti-periodic effect of this trivalent arsenical. This was done to enhance the evaluation of the sulfonamide therapy. Specimens of urine were sent to the laboratory for examination every other day and daily if the indication arose.

A number of different treatment schedules were used in the several cases treated. The general principle of heavier dosages on the first day or two followed by a reduced maintenance dosage, was adhered to in all cases. In series No. 1 the treatment periods varied from 4 to 8 days with four patients under treatment for 4 days, two for 5 days, two for 7 days, and two for 8 days. The average number of treatment days was 5.6. Total dosages varied from 24 to 40 gm., four patients receiving 24 gm., two 28, two 36, and two 40 gm.; average amount 30.4 gm. Sulfadiazine was used in all cases except one in which sulfathiazole was employed due to the complicating development of an urinary tract infection. In series No. 2, comprising 4 cases, treatment periods were 9 days in 1 case and 10 days in each of the other 3 cases. Here total dosages varied from 44 to 48 gm. The purpose of dividing treated cases into two series was to compare efficacy of longer treatment periods with that of shorter periods but since the number of cases in series No. 2 was so small such a comparison would have been of no statistical value. Relapse rates were therefore computed from the total number of cases making up the two series. Incidentally, the rates in the two series were approximately the same.

All patients were protected from exposure to direct sunlight.

Results. The treatment periods under the sulfonamides were uneventful in the great majority of the cases treated. One patient suffered severe emesis as a result of his sulfadiazine therapy. Vomiting was not alleviated by alkalization. It was felt that he retained only a small amount of the drug from each dose and he was therefore not included in the group of patients from which the relapse rate was computed. All others in both series were included.

In the 13 cases which were considered suitable for the study there were 3 relapses, or a relapse rate of 23%. In Case 13148, one of the 3 relapses, there was no recurrence of symptoms but there was a reversion of blood smears to positivity. This relapse occurred 79 days after completion of the sulfadiazine course. A course of 4 days sulfa-

diazine therapy at the time of the relapse caused the parasites to disappear from the peripheral circulation and to this date, 150 days since, there has been no symptomatic relapse and plasmodia have not reappeared in the blood. In Case 13206 there was a symptomatic relapse 33 days after completion of the sulfadiazine course and plasmodia were found to be present in the blood. At the beginning of the second course of sulfadiazine, then begun, emesis occurred with each dose and the drug was abandoned in favor of quinine sulfate. Of the 4 cases constituting series No. 2, one (Case 13297) suffered symptomatic relapse with reappearance of plasmodia in the blood 44 days after sulfadiazine therapy. An additional course of sulfadiazine was begun at once and continued for a period of 4 days. Abatement of symptoms and disappearance of plasmodia from the blood was the result. At this time, 68 days since, there has been no recurrence of fever and several blood smears have been negative.

FIGURE 3—SERIES NO. 1

Case No.	Type malaria used	Drug used	Days, treatment	Amount, gm.	Pre-treatment smears	Smears after treatment	Days since treatment	Relapse	Toxic reaction
13165	Q	Diazine	7	36	Pos.	Neg.	239	No	No
13148	Q	Diazine	8	40	Pos.	Neg.	248	Yes	No
13194	Q	Diazine	7	36	Pos.	Neg.	227	No	No
12503	Q	Diazine	5	28	Pos.	Neg.	198	No	No
13190	Q	Thiazole	8	28	Pos.	Neg.	264	No	No
13206	T	Diazine	5	28	Pos.	Neg.	204	Yes	Yes
12882	Q	Diazine	4	24	Pos.	Neg.	190	No	No
12501	Q	Diazine	4	24	Pos.	Neg.	179	No	No
13247	Q	Diazine	4	24	Pos.	Neg.	186	No	No

Ten patients originally constituted this series but 1 was omitted as explained in text. Patients in this series received from 4 to 8 days of sulfonamide treatment while those in series No. 2 received either 9 or 10 days of treatment each. It was intended to run the two series in order to compare efficacy of shorter and longer courses but small number of cases in series No. 2 would not permit statistical comparison of any value. Relapse rates are computed on basis of total number of patients in the two series.

T = Tertian. Q = Quartan.

FIGURE 4.—SERIES NO. 2

Case No.	Type malaria used	Drug used	Days, treatment	Amount, gm.	Pre-treatment smears	Smears after treatment	Days since treatment	Relapse	Toxic reaction
13300	Q	Diazine	10	48	Pos.	Neg.	124	No	No
13297	Q	Diazine	9	44	Pos.	Neg.	116	Yes	No
13323	Q	Diazine	10	46	Pos.	Neg.	73	No	No
13345	Q	Diazine	10	46	Pos.	Neg.	70	No	No

This series consisted of 4 patients. Treatment periods were 9 to 10 days and the total dosage of sulfadiazine was from 44 to 48 gm. There was 1 relapse in the 4 cases.

T = Tertian. Q = Quartan.

It will be noted in the accompanying tables that in no case did the drug fail to bring about a disappearance of plasmodia from the blood smears. Likewise, no patient failed to obtain complete symptomatic relief from this medication. Our experiences with sulfadiazine in the cases which suffered relapses, as well as those which did not, has led us to the practice of routine use of the drug in all cases but the exceptional ones in which it is not tolerated. The length of time required for control of paroxysms by use of sulfadiazine was found in our series to closely parallel that found by Coggeshall and co-workers[2] in a series of naturally acquired malarial cases.

It is conceivable that the implication of these observations could be of military significance, in that army personnel finding themselves in

isolated positions could employ this drug when contracting malaria. (We are in receipt of advice from local military-medical authorities that sulfadiazine tablets are replacing the sulfanilamide tablets which have been carried on the persons of soldiers in the field.)

The question of why some patients are permanently cured of the disease while others have relapses may possibly be answered at a later date by sulfadiazine blood level studies. So far as we have been able to learn this has not yet been undertaken. The above-described cure of relapsed cases with a second course of the drug should lead to further studies along this line and elicits the possibility of a treatment schedule which would employ a second course initially instead of only after a relapse. Both of these considerations are being investigated in cases currently under treatment by the author.

Summary. 1. The need for a new and effective anti-periodic are described, along with present obstacles in the use of quinine derivatives.

2. The author's independent discovery of the anti-malarial action of sulfadiazine, and of sulfathiazole as a point of predication, is considered.

3. A résumé of the work of Schwartz *et al.* indicates that sulfathiazole in their hands was effective as an anti-malarial in only about 50% of the cases.

4. The work of Coggeshall *et al.* with the sulfonamides, promin and sulfadiazine, in the treatment of naturally acquired and therapeutic malaria is reviewed. Results in the latter were inconclusive but were indicative that sulfonamides possessed an anti-malarial action in both naturally acquired and therapeutic malaria. Sulfanilamide was found to be without effect in human malaria.

5. Quartan malaria is used therapeutically much more widely than formerly, so that positive treatment results with sulfadiazine have also become more important.

6. Toxic and other untoward treatment complications in the use of sulfadiazine are described along with precautions to be observed.

7. Technique of administration and treatment schedules are discussed. The author's treatment results are described and tabulated.

8. Possible military significance of the author's treatment results is discussed.

Conclusions. 1. Sulfadiazine is an effective anti-malarial.

2. It is exhibited with a minimum of toxic reaction and is therefore taken by patients with less reluctance than is quinine.

3. The relapse rate, employing our dosage schedules and in therapeutic quartan malaria, has been established at 23%. The amount of treatment used appeared to have no relation to the relapse rate. Patients suffering relapses had had 5, 8, and 9 days of treatment respectively.

4. Relapses are controlled by a second course of sulfadiazine. No patient has had a second relapse.

5. The anti-malarial action of sulfadiazine could be used to advantage by the military in malarious areas.

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PROTHROMBIN STUDIES USING RUSSELL VIPER VENOM

VI. The Stability of the Thromboplastic-like Activity of Russell Viper Venom Under Various Conditions of Storage

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The use of Russell viper venom as a thromboplastic-like substance in Quick's test[4] for prothrombin clotting time has been advocated on the grounds that it possesses several advantages over thromboplastin derived from rabbit brain. Essentially the same relative results have been obtained with both thromboplastic agents.[3] The commercially available venom is convenient, quite uniform within and between batches, and, as originally packaged, stable for at least 5 years.[1] The present study deals with the stability of venom solutions under various conditions of storage.

Method. Considerable amounts of 2 batches of venom were secured.† A portion of each batch of venom was dissolved in water to make a concentration of 1 to 10,000. A water solution of venom from many ampoules of the same batch was pooled and then divided into 4 portions and stored as follows: 2 portions, 1 tightly stoppered and 1 lightly stoppered (the opening being sufficiently small to prevent appreciable evaporation and consequent concentration of the venom but sufficiently large to permit the access of air), were kept in the laboratory at room temperature, and the 2 other portions, 1 stoppered and the other lightly stoppered, were kept in a refrigerator.

At 2-week intervals throughout a period of 18 weeks, all 4 samples were tested for thromboplastic activity by determining the prothrombin clotting time of samples of normal venous blood. The method consisted in adding venom solution to oxalated plasma and then adding calcium chloride. The time required for the formation of fibrin flecks is the prothrombin clotting time.[2] All determinations were done in triplicate.

The balanced experimental design as indicated in the tables possessed several advantages. In addition to revealing the effects of storage conditions, com-

* On active service with the Armed Forces of the United States.

† Russell Viper Venom ("Stypven") was supplied by Burroughs Wellcome & Co. (U. S. A.) Inc., New York, N. Y.

Table 1.—Effects of Storage Conditions on the Thromboplastic Activity of Solutions of Russell Viper Venom* as Manifested in the Clotting Times of Normal Human Blood

TABLE 1.—EFFECTS OF STORAGE CONDITIONS ON THE THROMBOPLASTIC ACTIVITY OF SOLUTIONS OF RUSSELL VIPER VENOM* AS MANIFESTED IN THE CLOTTING TIMES OF NORMAL HUMAN BLOOD

	Batch No. 191A—Expiration date: March 4, 1941										Batch No. 200A—Expiration date: June 17, 1941									
	Control (freshly prepared each time)		Refrigerated (tightly stoppered)		Refrigerated (lightly stoppered)		Room temperature (tightly stoppered)		Room temperature (lightly stoppered)		Control (freshly prepared each time)		Refrigerated (tightly stoppered)		Refrigerated (lightly stoppered)		Room temperature (tightly stoppered)		Room temperature (lightly stoppered)	
Subjects:	H	P	H	P	H	P	H	P	H	P	H	P	H	P	H	P	H	P	H	P
Interval	(sec.)		(sec.)		(sec.)		(sec.)		(sec.)		(sec.)		(sec.)		(sec.)		(sec.)		(sec.)	
Freshly prepared	19.8	17.2	19.8	17.2	20.2	17.2	20.5	16.0	19.6	16.0	19.8	15.8	19.8	15.8	19.8	17.0	19.0	15.8	19.4	16.4
	20.8	17.0	20.8	17.0	20.4	17.4	21.0	16.2	19.8	16.2	20.0	16.2	20.0	16.2	20.4	17.4	18.2	15.6	19.6	16.6
	20.4	17.4	20.4	17.4	19.6	17.6	21.2	16.6	20.2	17.0	20.2	16.4	20.2	16.4	19.6	16.6	19.2	15.9	20.2	16.8
1 week	17.5	18.2	19.4	21.0	20.2	19.5	17.8	20.0	20.0	23.0	20.2	20.8	19.2	21.8	19.2	19.8	19.4	21.1	19.2	20.5
	17.8	19.0	20.0	21.6	20.4	20.3	18.0	21.0	20.5	23.2	19.8	20.6	20.1	20.9	19.0	20.6	20.4	21.4	19.4	20.7
	18.2	19.0	20.4	22.0	20.6	20.5	18.5	20.8	20.6	23.3	20.8	20.1	19.4	21.0	19.4	20.8	19.5	21.7	20.0	21.2
2 weeks	17.5	18.1	15.9	19.0	17.9	17.0	16.0	17.6	16.0	16.8	17.0	16.9	17.2	16.0	16.2	16.4	15.5	15.8	16.2	16.1
	17.8	18.6	16.4	20.0	17.1	17.1	16.2	18.2	16.1	16.4	17.2	17.6	16.6	15.2	16.1	16.2	16.0	16.7	16.4	16.4
	18.2	18.7	16.2	20.0	17.5	17.9	16.7	17.6	16.9	16.5	17.1	17.1	16.0	16.0	15.8	16.5	16.1	16.2	16.1	16.2
4 weeks	17.5	17.0	16.8	16.8	17.0	16.5	16.5	16.5	16.1	17.2	16.8	17.5	16.2	16.2	16.2	16.9	16.4	17.0	15.8	17.1
	17.8	17.8	17.0	16.9	17.1	16.7	17.0	17.0	16.0	17.4	16.9	17.4	16.4	15.8	16.3	17.2	16.6	16.3	16.0	17.6
	17.6	17.5	17.1	17.4	16.9	16.8	17.1	16.4	16.4	17.0	17.2	17.8	16.0	16.4	16.5	17.1	16.2	16.2	16.3	17.4
6 weeks	19.5	19.2	19.4	20.2	20.0	21.8	20.1	18.9	21.0	24.0	18.6	19.0	20.1	18.9	22.2	19.0	19.6	18.9	23.1	19.6
	20.0	19.0	20.1	20.4	19.6	21.6	20.6	18.7	20.8	23.2	19.4	19.1	20.4	19.4	22.0	19.2	19.5	19.4	22.8	20.2
	20.0	19.4	20.4	20.6	20.4	22.0	19.5	19.2	21.2	22.8	18.8	19.4	20.2	19.5	22.4	19.1	20.2	19.1	22.9	20.3
8 weeks	21.8	19.5	20.5	19.0	23.1	22.5	21.9	19.2	23.4	23.2	19.8	18.9	20.8	20.1	22.9	22.8	19.2	19.6	22.1	19.6
	21.0	19.8	21.4	19.1	22.9	22.9	22.1	19.6	23.2	22.9	19.9	19.6	21.6	21.2	22.8	23.0	20.4	19.8	22.0	20.0
	21.3	19.9	21.6	19.3	22.6	22.3	21.5	18.1	22.9	23.0	19.6	19.2	20.5	21.0	23.1	22.7	20.2	19.5	22.4	19.9
10 weeks	21.2	21.2	20.8	20.6	21.0	22.1	20.4	21.5	36.1	32.4	21.6	21.2	20.6	20.4	21.0	20.6	21.0	20.5	21.2	26.5
	21.8	21.2	20.6	20.8	20.5	21.9	21.0	21.6	36.6	32.6	21.6	21.0	20.8	20.2	21.2	20.5	21.2	20.6	21.4	27.0
	21.2	21.3	20.9	20.8	20.6	21.7	20.6	21.4	37.0	32.0	21.4	21.6	21.1	20.3	21.3	20.8	21.4	20.3	21.6	26.9
12 weeks	23.0	21.0	22.0	19.4	23.0	20.5	22.9	20.0	33.5	30.0	21.5	18.5	19.6	19.1	20.2	20.0	20.7	19.2	20.6	28.0
	22.9	20.6	21.2	18.9	22.8	20.3	22.8	19.6	33.0	29.7	21.4	18.8	20.1	19.2	20.5	20.2	20.1	19.0	20.9	28.5
	23.2	20.7	21.7	19.0	22.5	20.8	22.4	19.3	33.7	30.2	21.0	19.0	20.4	18.8	20.1	19.8	19.9	19.7	21.4	27.7
14 weeks	20.1	17.8	20.1	17.5	20.5	17.5	22.1	17.8	37.0	25.8	20.0	15.5	20.8	16.6	19.6	19.8	20.2	17.5	22.0	20.0
	21.0	16.0	20.0	18.0	21.2	17.9	22.1	18.0	37.5	24.9	19.6	15.5	20.0	16.1	20.1	19.2	20.8	17.6	22.4	20.1
	20.8	15.8	19.6	17.9	21.0	17.0	22.0	18.1	36.0	25.0	20.2	14.8	20.2	17.0	20.2	19.0	21.0	17.1	22.1	20.6
16 weeks	21.5	18.5	20.6	19.4	19.6	20.0	22.4	19.0	32.0	25.0	19.8	18.2	19.5	18.0	19.0	18.6	20.4	18.5	24.0	19.5
	21.5	19.0	20.4	19.0	20.1	19.6	22.6	19.2	32.4	25.2	20.4	17.7	20.0	18.5	19.4	19.0	20.8	18.0	25.2	20.0
	21.2	19.2	20.0	19.5	20.0	19.2	22.5	19.5	34.0	25.4	19.6	18.0	20.1	18.6	19.3	18.7	20.6	18.6	24.6	20.6
18 weeks	21.8	22.5	22.5	21.6	21.6	21.1	23.4	22.0			20.2	21.2	20.8	17.5	19.6	17.0	20.4	21.0	27.5	
	22.1	22.4	23.0	21.8	21.4	21.4	23.1	21.4			20.8	21.7	21.0	17.0	19.9	17.2	21.2	20.8	28.0	
	22.0	23.0	22.6	21.9	21.5	21.9	23.0	21.9			20.5	21.5	20.9	17.2	20.0	17.5	21.0	21.1	28.2	

* Supplied as "Stypven" Russell viper venom by Burroughs Wellcome & Co., New York.

parisons could be made between the 2 different batches of venom. Since it was desirable to have blood of as constant composition as possible, the same 2 persons furnished blood for the entire study. By using 2 individuals, 1 served as a control for the other. Both were healthy young adults. However, since even the blood of healthy individuals might vary, venom from freshly opened ampoules served as standards of control at each 2-week period. More than 650 determinations were performed in the course of this study.

Results. The results are given in Table 1. Inspection of the data reveals the importance of control determinations, as periodic fluctuations in the prothrombin times of both subjects were found to occur. In order to correct for these fluctuations the average control values were subtracted from the corresponding average experimental values of each of the 4 stored samples and the results plotted as in Figure 1.

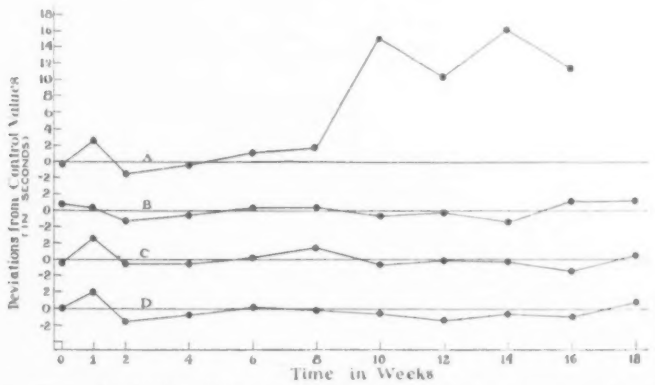

FIG. 1.—Thromboplastic activity of samples of Russell viper venom under different conditions of storage. The differences in prothrombin clotting times between stored samples and the control values are plotted against the duration of storage. Curve A—lightly stoppered, room temperature. Curve B—tightly stoppered, room temperature. Curve C—lightly stoppered, refrigerator temperature. Curve D—tightly stoppered, refrigerator temperature.

It was found that storage at refrigerator temperatures in stoppered or lightly stoppered containers as well as storage in stoppered flasks at room temperature, did not significantly affect the thromboplastic-like activity. The sample which was left lightly stoppered at room temperature showed no change for the first 8 weeks. At this point, however, the activity suddenly decreased very rapidly as indicated by the increased prothrombin time curves. The suddenness and the completeness of the change is surprising, as it would generally be expected that the decomposition would either occur soon after exposure or that it would be a more gradual process.

Summary. 1. Solutions of Russell viper venom maintained their thromboplastic-like activity unimpaired when stored in stoppered or lightly stoppered containers at refrigerator temperature or in stoppered containers at room temperature for the duration of the experiment (126 days).

2. Solutions of Russell viper venom suffered sudden decomposition after about 2 months exposure to the air at room temperature.

3. The prothrombin clotting times of two healthy normal individuals underwent periodic fluctuations over a period of 18 weeks.

We wish to express our appreciation for the assistance and coöperation of Dr. Louis Bauman in whose laboratory this work was carried out, and to Miss Mary Hamlin for her assistance.

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THE EFFECT OF ARSENIC (FOWLER'S SOLUTION) ON ERYTHROPOIESIS*

A Contribution to the Megaloblast-Normoblast Problem

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Prior to the discovery of liver therapy, arsenic was a common medication in the treatment of pernicious anemia, although no definite benefit from its use could be proved. Minot and Lee[12] believed that the drug acted as a stimulus to the bone marrow, though in larger amounts it appeared to cause destruction of this tissue. Stockman and Charteris[17] studied the action of arsenic on the bone marrow in man and in animals and concluded that there was an increase in the number of "leukoblastic" cells and a marked hyperemia and atrophy of the fat. There were little or no changes in the number or morphologic character of the "erythroblastic" cells. Evans[4] conceived the action of arsenic in pernicious anemia as one of "whipping up" an already overstimulated hemopoietic tissue, thus hastening its final collapse rather than promoting any real progress. It is well known that arsenic and its compounds are destructive to bone marrow as well as a protoplasm poison. Dustin[2] studied the effect of arsenic on the chromatin as it passes through certain phases and noted a nuclear hypersensitivity to the drug (karyoklasia) which occurs as the nucleus approaches a phase of condensation, either chromosomal or prepyknotic.

Tempka and Braun[18] reported the effect of arsenic therapy on a pernicious anemia patient who was observed for a period of 45 days. The patient received daily 150 mg. of arsacetin by mouth and 5 mg. acidum arsenicosum subcutaneously for the first 24 days. The blood findings before treatment were hemoglobin 37%, erythrocytes 1.25 mil., leukocytes 5100 and color index 1.5. Following arsenic therapy there were erythrocytes 3.21 mil., hemoglobin 72%, leukocytes 5900, and color index 1.1. After 17 days of arsenic, the bone marrow showed the

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promegaloblasts unchanged, the other forms of megaloblasts somewhat reduced and an increase in the number of pronormoblasts and normoblasts. The pathologic stab neutrophiles were increased. Similar findings were observed in the sternal marrow 45 days after arsenic therapy. Rohr[15] reported the effect of arsenic on the sternal marrow of 2 cases of pernicious anemia. One case who received 1 to 7 mg. acidum arsenicosum daily for 7 days showed an actual decrease in the hemoglobin percentage and in the number of erythrocytes. Sternal marrow aspiration on the 7th day of arsenic therapy revealed marked changes

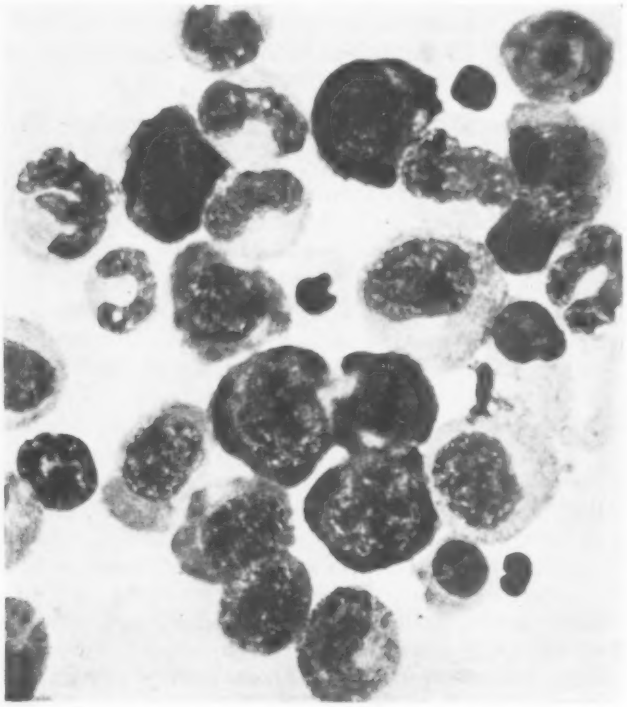

FIG. 1.—Bone marrow, Case 1. Showing megaloblasts and pathologic granulopoiesis. (× 1100.)

in the megaloblastic tissue. Their nuclei were swollen and misshapen and extreme karyorrhexis (karyoclasia) with many bizarre nuclear protuberances and large nuclear bits was a common observation. Arsenic was now discontinued and followed by the intramuscular injection of potent liver extract. The bone marrow examination 11 days later revealed a macronormoblastic regeneration and a disappearance of the pathologic erythropoiesis due to arsenic. Exactly the same changes were observed in a second case. According to Rohr, arsenic brings about a pathologic stimulation of the megaloblasts which consist of a hastening of cytoplasm ripening, nuclear destruction and

nuclear extrusion. This may produce a megalocytic remission in the peripheral blood, but normalization of the pathologic bone marrow never occurs. The effects of arsenic in the other anemias stimulates normal erythropoiesis. On the other hand, Naegeli[13] as late as 1935 maintained that arsenic therapy was capable of producing unquestionable remission in pernicious anemia. Koller[9a] studied the sternal marrow of a pernicious anemia patient who was treated with acidum arsenicosum (10 daily doses—0.002 to 0.010 gm.) and estimated the length of time it takes for basophilic megaloblasts to become megalocytes. Before treatment, the marrow was megaloblastic in type, on the 6th day of arsenic medication, the polychromatophilic megalo-

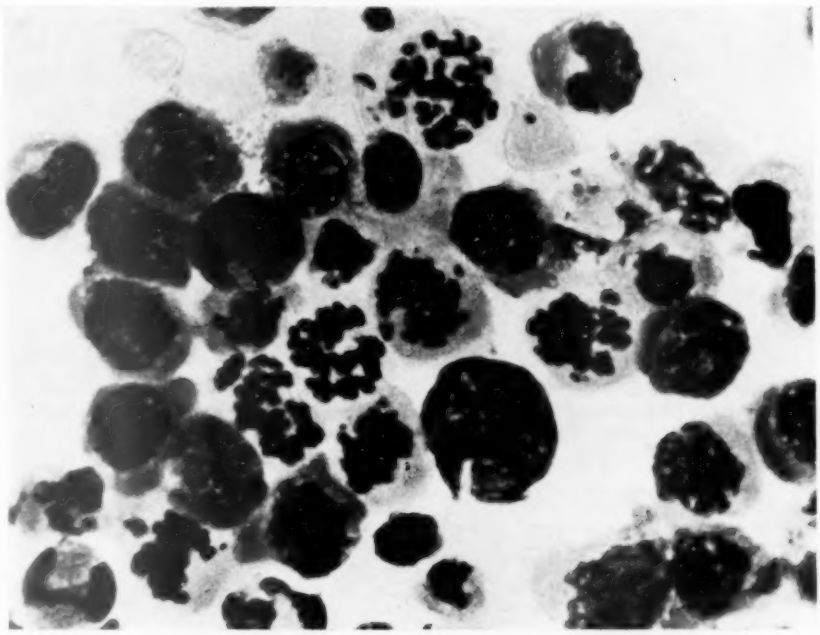

FIG. 2.—Bone marrow, Case 1. After 4 days of arsenic medication. Note the marked karyorrhexis of megaloblasts. (Arsenic sensitive.) (× 1100.)

blasts were more than double in number and on the 10th day orthochromatic megaloblasts were increased approximately 11 times. From these studies he estimated it takes about 10 to 12 days for the maturation of megalocytes in contrast to the 3 to 4 days for normocytes following liver therapy. Koller is aware of the fact that arsenic therapy is unphysiologic.

Ehrlich[3] maintained that the erythroid cells of the primitive prehepatic generation of erythrocytes in the embryo were identical to the megaloblastic generation of red cells characteristic of pernicious anemia during a relapse. He believed in an embryonal reversion of erythropoiesis in pernicious anemia. Recent[7] studies indicate that the red

cells of the primitive generation in the embryo, though morphologically similar, are not identical with the megaloblastic regeneration of pernicious anemia.

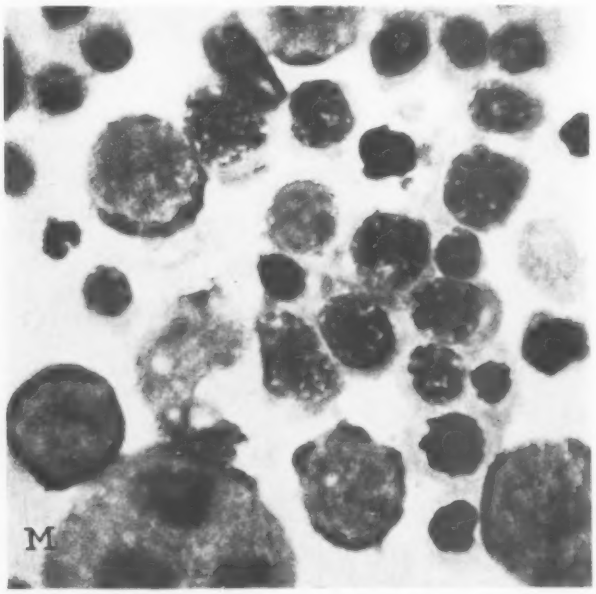

FIG. 3.—Bone marrow, Case 1. Seventy-two hours after 10 cc. of intramuscular liver extract (20 units). Showing conversion to normal erythropoiesis. (Normoblastic.) M. multipolar mitosis. (× 1100.)

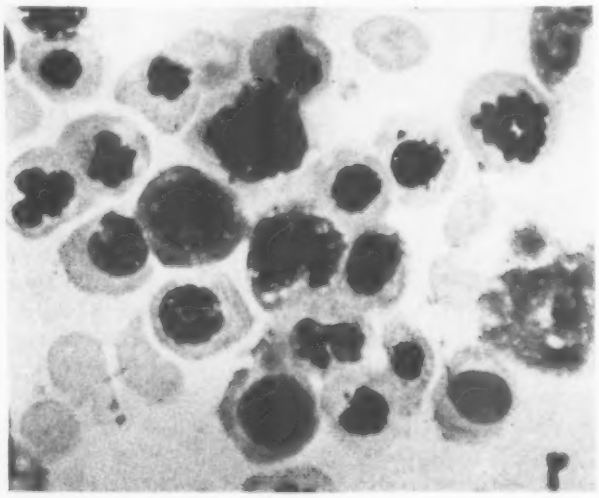

FIG. 4.—Bone marrow, Case 2. After 6 days of arsenic therapy showing maturation of megaloblasts to acidophilic stage. (× 1100.)

The theory that the megaloblast is a normal erythroid cell which requires the "liver factor" for its maturation to a normoblast has enjoyed a widespread popularity.[1,6,14] On the other hand, the contention that the megaloblast is a pathologic cell and that the hemopoietic principle exerts its action in part by eliminating and inhibiting the formation of megaloblasts is supported by clinical experiments.

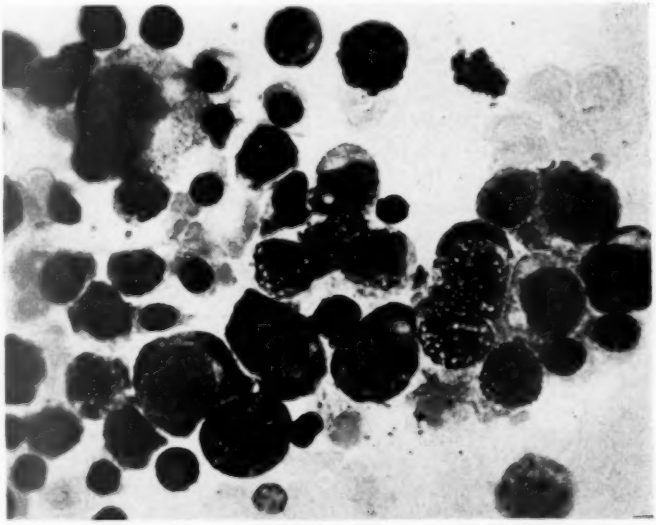

FIG. 5.—Bone marrow from case of sickle-cell anemia. Showing marked erythroid immaturity (pronormoblasts) and normoblasts unaffected by arsenic after 17 days of medication. (Arsenic resistant.) (× 1100.)

Methods. The method used for obtaining and studying bone marrow has been described in detail elsewhere.[10] To state it briefly—1 cm. of sternal marrow is aspirated through a dry modified 16-gauge needle into a dry syringe and placed immediately into a paraffin-lined tube containing a small amount of powdered heparin, an anticoagulant; the marrow material is transferred to a Wintrobe hematocrit tube; centrifuged 5 minutes at about 2000 r.p.m.; the layers into which the marrow separates (erythrocytes, bone marrow cells, plasma, and red and yellow fat) are recorded; the bone marrow cell layer is mixed with an equal volume of plasma in a paraffin chamber; and films of this mixture are made, stained with May-Grunwald-Giemsa dyes, and studied microscopically for cell distribution and types.

Studies of the peripheral blood drawn just prior to the sternal aspiration are made in all cases. This includes counts of erythrocytes, leukocytes and platelets; hemoglobin and hematocrit determinations, mean corpuscular volume, hemoglobin and hemoglobin percentage; sedimentation rate, icteric index, reticulocyte count and differential leukocyte counts.

Arsenic in the form of Fowler's solution (Liquor Potassii arsenitis corresponding to 1% AS_2O_3 was administered in milk, beginning with 4 minims 3 times a day and increasing the dosage 3 minims each successive day until the dosage of 15 minims 3 times a day was reached, or until toxic symptoms of the drug were manifested. Some of these are hyperesthesia of the oral mucous membrane, sensation of esophageal irritation, severe gastro-intestinal disturbances such as anorexia, cardialgia, nausea, vomiting and diarrhea, puffiness of the eyelids and skin rashes. The drug was then discontinued or

the dosage was gradually decreased. The toxic effects of arsenic were observed in all cases of pernicious anemia, in some instances as early as the 3d day following medication.

Seven cases of pernicious anemia were studied before treatment after the administration of arsenic and following intramuscular liver injections. Table 1 shows that those cases with a severe anemia and marked megaloblastic hyperplasia of the bone marrow are extremely sensitive to arsenic. Although there was no improvement in the anemia, a reticulocytosis as high as 31% was observed in the peripheral blood. The bone marrow, as early as the 4th day, revealed marked toxic and destructive changes in the megaloblastic tissue consisting of various phases of karyorrhexis such as swollen and misshapen nuclei, bizarre nuclear protuberances, nuclear fragments, nuclear destruction and nuclear extrusion. Mitotic figures were infrequent. There was a definite and pronounced maturation of megaloblast to the acidophilic stage. There were no morphologic indications that megaloblasts matured to normoblasts.

In pernicious anemia marrow after arsenic treatment the pronormoblasts and later stage cells are intact and they may even be adjacent to disintegrating megaloblasts. Following intramuscular liver therapy there is a tremendous proliferation of normoblasts in as short a period as 24 hours. This is followed by a gradual improvement in the anemia in the peripheral blood. Obviously the arsenic-sensitive pathologic megaloblastic tissue which is in a karyorrhectic condition cannot take part in the normoblastic transformation and normalization of the bone marrow in pernicious anemia following liver treatment.

Arsenic was administered to normal controls, cases of cirrhosis of the liver, sickle-cell anemia, erythroblastosis, polycythemia vera, chronic myeloid leukemia, carcinoma of the stomach and chronic hemorrhagic anemia (Table 2). In these various clinical disorders in which the bone marrow consists of a moderate to marked erythroid hyperplasia and immaturity no significant degenerative changes of the erythropoietic tissue were observed. In spherocytic jaundice and refractory anemia with a macrocytosis following the administration of arsenic, karyorrhexis in the polychromatophilic and acidophilic normoblasts in the bone marrow was a conspicuous feature. In none of the bone marrows were the pronormoblasts affected by arsenic. These findings are additional evidence for the separation of the pathologic megaloblastic series from the normoblastic series of erythrocyte regeneration.

Jones,[8] in a comprehensive article has discussed in detail the morphologic, physiologic, chemical and biologic distinction of megaloblasts. This investigator believes the morphologic aspects of erythropoiesis have been unduly neglected and have resulted in calling all immature erythroid elements, "megaloblasts," whether they are seen in normal bone marrow, in that of various clinical conditions, or in bone marrow of certain laboratory animals.

In my own study of 2325 sternal marrows, megaloblasts were observed almost entirely in patients with anemias due to a deficiency

TABLE 1.—CASES OF PERNICIOUS ANEMIA TREATED WITH ARSENIC AND LIVER

Before Treatment

TABLE 1.—CASES OF PERNICIOUS ANEMIA TREATED WITH ARSENIC AND LIVER

Before Treatment							
Case No.	1	2	3	4	5	6	7
Erythrocytes, millions	1.14	1.30	1.47	2.34	2.46	2.60	2.90
M.C. volume, C.M.	140	145	122	130	138	120	121
Reticulocytes, %	1.0	0.5	0.5	0.5	0.1	0.5	0.5
Bone marrow, type	Megaloblast	Megaloblast	Megaloblast	Megaloblast and normoblast	Megaloblast and normoblast	Megaloblast and normoblast	Megaloblast and normoblast
After Arsenic							
Days on arsenic	4	6	8	14	6	7	8
Erythrocytes, millions	1.40	1.20	1.63	2.90	2.39	2.50	2.80
Reticulocytes, %	19.0	31.0	18.0	12.0	8.0	3.5	5.0
Bone marrow (karyorrhexis, %)	90.0	88.0	72.0	86.0	42.0	59.0	65.0
After Liver							
Days after liver	2	3	13	....	3 mos.	2 mos.	16 days
Erythrocytes, millions	1.20	1.17	2.34	....	4.70	4.30	3.80
Reticulocytes, %	22.0	37.0	6.0	....	0.5	0.5	1.0
Bone marrow (type)	Normoblast and some karyorrhexis	Normoblast	Myeloid and normoblast	....	Normal	Normal	Myeloid and normoblast

TABLE 2.—VARIOUS CLINICAL DISORDERS TREATED WITH ARSENIC

	Before					Following arsenic			
Blood disorder	Hemoglobin (gm.)	Erythrocytes (mill.)	Reticulocytes (%)	Bone marrow (type)	Days on arsenic	Hemoglobin (gm.)	Erythrocytes (mill.)	Reticulocytes (%)	Erythroid cells affected (karyorrhexis)
Normal controls (4)	..	..	..	Normoblast	8–18	13.0	4.64	0.6	None
Cirrhosis of liver (macrocytic)	12.0	3.20	0.5	Pronormoblast, normoblast	10	10.8	2.90	0.5	Normoblast (slight)
Refractory anemia (macrocytic, 2)	7.4	1.86	0.5	Pronormoblast, normoblast	21	7.4	2.25	6.0	Normoblast (marked)
Sickle-cell anemia (macrocytic, 2)	9.5	2.95	20.0	Pronormoblast	10–17	9.5	2.68	12.0	None
Erythroblastosis (macrocytic)	10.0	2.26	40.0	Normoblast	16	6.5	1.61	5.0	Normoblast (mod.)
Spherocytic jaundice (normocytic, 2)	7.9	2.78	20.0	Pronormoblast, normoblast	11–15	8.0	2.77	25.0	Normoblast (marked)
Polycythemia vera	19.0	7.79	1.0	Pronormoblast, normoblast	14 (Venesection)	15.5	5.64	1.5	Normoblast (mod.)
Chronic myeloid leukemia (normocytic)	8.5	2.90	1.0	Normoblast	30	8.25	3.30	4.5	Normoblast (slight)
Ca. of stomach (microcytic)	4.5	2.61	1.0	Pronormoblast, normoblast	14	4.5	2.50	1.5	None
Chronic hemorrhage (microcytic, 2)	5.0	3.01	0.5	Pronormoblast, normoblast	13–17	4.4	3.35	1.0	Normoblast (slight)

of liver principle. Rohr[16] has had a similar experience in a series of more than 1800 sternal marrow examinations.

In pernicious anemia, liver extract and arsenic affect the bone marrow differently. The physiologic effect of liver extract results in the conversion or transformation of a megaloblastic marrow to a normoblastic one in 24 to 72 hours. The pathologic effect of arsenic causes no delay in the conversion or transformation to a normoblastic marrow following intramuscular liver treatment. The pathologic cell of the granulocytic series and the megakaryocytes are unaffected by arsenic. Following liver therapy the abnormal granulopoietic tissue and the megakaryopenia gradually returns to normal. It is interesting to note that arsenic does not affect the pronormoblasts seen in various clinical conditions presenting marked erythroid hyperplasia and immaturity.

The implications are great that the effect of liver extract is one of eliminating the pathologic megaloblasts and suppressing their further multiplication.[5]

The resultant reticulocytosis following arsenic therapy is due to the maturation of megaloblasts to reticulated megalocytes, since there is no morphologic indication that megaloblasts are maturing to normal reticulocytes. Normally following liver therapy in pernicious anemia, megaloblasts complete their maturation as reticulated and mature megalocytes. The resultant reticulocytosis is composed of reticulated megalocytes and normal reticulocytes. According to Jones[9] this not only indicates the reëstablishment of normal erythropoiesis, but also that the marrow is being purged of its pathologic red cell series, the megaloblasts.

It is unfortunate that blood cells under embryonic, normal adult and pathologic conditions all have their nuclei pass through a pyknotic stage before becoming a reticulocyte. Hence "tagging" the megaloblasts with arsenic makes it easier to trace cellular maturation which were followed with difficulty after liver extract alone.

The mechanism by which a megaloblastic marrow is rapidly transformed or converted into a normoblastic one is an unsettled question. Normal bipolar mitosis does not explain the rapid and dramatic conversion. Koller[9a] has suggested that young multinucleated megaloblast cells may subdivide and heteroplastically form a quantity of normoblasts. Since the arsenic-sensitive megaloblasts are not transformed into normoblasts, the finding of multipolar mitoses and multinucleated erythroid[5,11] cells which are frequently seen in pernicious anemia marrows may play a rôle in the rapid conversion to a normoblastic type.

The effect of arsenic on the normoblasts in spherocytic jaundice is probably the result of the fundamentally abnormal type of erythropoiesis which produced the spheroidocytes. The toxic action of the drug on the erythroid cells in the case of refractory anemia cannot be satisfactorily explained.

Conclusions. 1. The megaloblasts in the bone marrow of patients with pernicious anemia during relapse are arsenic sensitive and the pronormoblasts as seen in normal bone marrow and in the bone marrow of most cases of anemias with an erythroid immaturity are arsenic resistant.

2. The "liver principle" can exert its physiologic effect on the bone marrow in pernicious anemia in the presence of a karyorrhectic megaloblastic tissue which presumably is in a poorly functional condition due to the toxic effect of arsenic.

3. The action of the hemopoietic principle is probably to effect the elimination (maturation) and suppression of the megaloblasts in the bone marrow. It is not clear as to how liver extract effects a stimulation of the normoblastic tissue. It is clear that arsenic has no such effect.

4. The arsenic serves as a convenient agent to separate the pathologic reticulocytes originating from the megaloblasts from those derived from normoblasts. When the latter appear, the anemia is corrected.

It is especially important for the reader to keep evidence separate from deduction in this article. The interesting observations might perhaps be brought into line with other views as to the rôle of the megaloblast in erythrypoiesis.—EDITOR.

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A STUDY OF HEMOGLOBIN METABOLISM AND HEMATOLOGY IN A CASE OF CONGENITAL HEMOLYTIC JAUNDICE DURING (A) CLINICAL CRISIS, (B) REPEATED TRANSFUSIONS, AND (C) BEFORE AND AFTER SPLENECTOMY

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THIS case is reported because of the continuous period of study before splenectomy, through the course of a clinical crisis with evidence of liver dysfunction, during repeated blood transfusions, and

finally, after splenectomy. Similar studies of hemolytic jaundice before and after splenectomy have appeared in the literature[7,8,17] but none covers all these various phases.

There are several reports of clinical or pathologic evidence of liver complications occurring during the course of hemolytic jaundice of various types.[3,15,19] The clinical picture includes: (1) liver enlargement; (2) repeated attacks of nausea, vomiting, fever, and progressive jaundice, with upper right quadrant pain in the presence or absence of gall stones; (3) a direct van den Bergh reaction in the serum, or one changing from indirect to direct during the acute episode. The pathologic changes observed include: (1) marked enlargement of the liver with no histologic reports; (2) a perilobular congestion of characteristic type; (3) some degree of parenchymal degeneration, varying from mild with recovery to severe with terminal acute yellow atrophy.[2,3,4] The case reported by Farrar[3] showed moderate liver degeneration with recovery following splenectomy. The clinical condition was aggravated by transfusions. They suggest that an acute liver failure is one of the pathogenic mechanisms of the clinical crises observed in hemolytic anemia.

The effect of transfusions on the course of blood destruction in sickle cell anemia and in congenital hemolytic jaundice has been extensively studied by Josephs.[9] He observed inhibition of the hemolytic process with whole blood and plasma transfusions,[8] as well as with pig plasma.[17] Others have noted the dramatic results from transfusions in the so-called Lederer's acute hemolytic anemia.[1,11] Transfusions were long used as the mainstay in management of acute phases of congenital hemolytic anemia also,[5,13] but more recently warnings have been published against their use, as liable to aggravate the condition and even prove fatal.[3,10,14]

There is adequate evidence in the literature for the value of splenectomy in congenital hemolytic anemia. However, recent publications[4,6,10] indicate that under certain circumstances splenectomy may be of no value in this condition, and the patients die. Some of these show a specific type of liver change characterized by perilobular congestion.

Case Report. J. H., white male, 15 years of age, was admitted to Charity Hospital of New Orleans (L. S. U. Med. Serv.) 12-10-40, complaining of anorexia, URQ pain, fever, jaundice, and enlarged spleen. He had apparently had no complaints up to 2 months previously, when he began having chilly sensations followed by malaise and anorexia. Later it was noticed he had fever and jaundice. He was nauseated and vomited several times. No abnormality was noted about the bowel action or the stools. The fever and vomiting continued and pain in the upper right abdomen occurred. He became delirious in a few days. His condition was thought to be due to gall bladder disease, and he was taken to a private hospital. On admission, the family stated that he had a very low red blood count. He continued to have high fever and the enlargement of the spleen was noted at this time. He stopped vomiting and regained his appetite, but continued to have fever. Following 5 blood transfusions over the course of his 19-day stay in that hospital he improved sufficiently to return home. He remained at home until the present admission, feeling well and gaining strength up to 1 week ago, at which time

he again began to have fever, lose his appetite and feel nauseated. He also began to have pain and soreness in the upper right quadrant of the abdomen.

His past history was irrelevant except that he had had a chronic ulcer of the middle third of the left leg several years previously which resisted treatment, but finally healed after a period of 3 months.

His family history was significant in that one brother had had an attack similar to that of the patient's after acting as a donor for him on his previous hospitalization. This brother was examined and found to have a large spleen, and has since been operated on. At the same time, the father was noted to have slightly icteric scleræ, and examination revealed a moderately enlarged spleen. His red blood cell fragility was increased, 0.6 to 0.38 (control 0.42 to 0.32).

Physical Examination. The temperature was 103.4° F., pulse 130, respiration 24, blood pressure 120/68. He was normally developed, but quite emaciated. His skin was hot, dry, and slightly jaundiced. The scleræ were definitely jaundiced. Lungs were clear, and heart negative except for the tachycardia and a systolic murmur at the apex. The abdomen was flat with a bulge in the left flank. Here a firm, notched, tender mass with rounded borders was felt, and thought to be the spleen. The liver was palpable 2 fingers below the costal margin on deep inspiration and was quite tender. On the left leg, one-third of the way from the ankle on the anterior surface was a pigmented scar measuring 5 x 3 cm.

Laboratory Reports. RBC, 2.72 million; MCV, 83μ^3; MCH, 32$\gamma\gamma$; RBC fragility, 0.6 to 0.34; control, 0.42 to 0.32. About 10% spherocytes were present. White blood cells, 11,200; neutrophils, 60; lymphocytes, 40%. Blood Type II. Urine amber, acid, sp. gr. 1.030, alb. 0, sugar 0, bile 0; microscopic: occasional hyaline and fine granular casts, and occasional white blood cells. Icterus index, 18 units. Blood Kolmer and Kline, negative. Roentgen ray of lungs and heart, negative. Gall bladder spot plates showed multiple stones in region of gall bladder and cystic duct.

Clinical Course. 12-13-40, transfusion 500 cc. cross matched blood, type unknown. 12-16-40, much improved, temperature normal. 12-20-40, ambulatory. 12-21-40, fever 100.2° F., jaundice increasing, complaining of pain in RUQ. Thought to be having a mild crisis. 12-24-40, intravenous hippuric acid liver function test, 0.59 gm. (the intravenous injection of sodium benzoate was immediately followed by nausea, vomiting, and urticaria which subsided immediately following 5 min. of epinephrine intramuscularly).

Transfusions were given: 1-7, 300 cc. Type IV; 1-12, 350 cc. Type II; 1-18, 350 cc. Type II.

The stercobilin and urobilin excretions were measured from 12-16 onwards and are illustrated in the accompanying chart. Following the initial rise of the RBC level, because of the increasing blood destruction as evidenced by the pigment excretion, and the ultimate fall of RBC, reticulocytes, and hemoglobin, coupled with progressive enlargement of the spleen which became more tender, it was felt that further management of this type was not indicated, and that surgery was necessary. Splenectomy was carried out by Dr. J. D. Rives of the L. S. U. Surgery Department on 2-10. It was noted at operation that besides the large spleen there were 10 or 12 small accessory spleens in the hilar region. All of them were removed. Immediately following splenectomy, 900 cc. of cross matched blood were given. That afternoon the RBC level was 4.45 million. The immediate postoperative course was marked by a sharp rise of temperature which subsided rapidly. The van den Bergh reaction was direct. The laboratory data are shown in the accompanying chart. (Fig. 1.)

The spleen weighed 1150 gm. and had 4 infarcts, 3 old ones and 1 more recent.

Discussion. The crises of hemolytic jaundice may be characterized by the prominence of various features. Gripwall[6] describes them as (1) thermic; (2) abdominal; (3) essential blood crises. The mechan-

ism of the so-called thermic crises, if they exist divorced from other phenomena, is not clear. His description of the abdominal crises fits those observed by ourselves and others, and which we feel, in agreement with Farrar, to be associated with changes in liver function and structure and not necessarily with increased blood destruction. Further study is necessary in those instances simulating appendicitis as happens in sickle cell anemia. The essential blood crises are the hemoclastic type producing severe, even fatal anemia. It is possible that

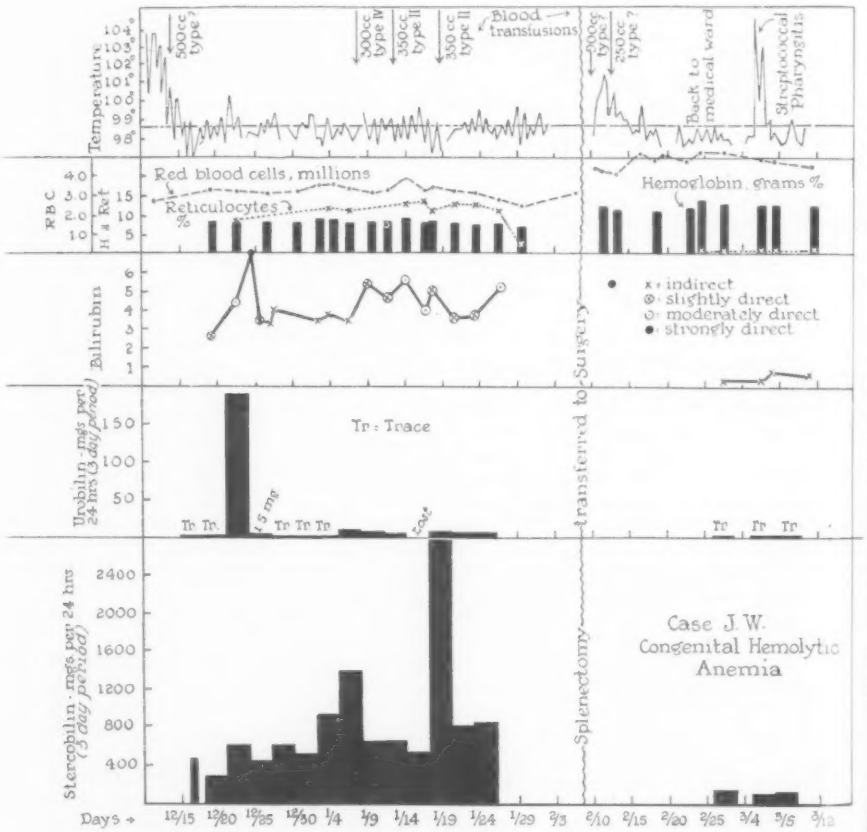

FIG. 1.—Laboratory data on case of congenital hemolytic jaundice.

these various clinical pictures are delineated by predominant manifestations of essentially the same mechanism. Not enough is known about the detailed pathologic physiology of the disease to make any definite statement as to the nature of this mechanism.

When this patient was first seen he presented a picture of hepatitis with splenomegaly. The history and examination of the father who was at the bedside immediately suggested congenital hemolytic jaundice in crisis. This occurrence of hepatitis in hemolytic anemia has

not been sufficiently studied. (The term "hepatitis" is used to describe the clinical picture. We have no data on the actual pathologic anatomy.) We have repeatedly observed it in sickle cell anemia. Frequently the patient is immediately given a transfusion, as was done in this instance, but that spontaneous recovery[5] from the episode does occur is also obvious from the second such attack observed in this case. Josephs calls attention to the possibility of confusing spontaneous variation with the apparent therapeutic effect of a transfusion.[7] Gairdner[5] claimed that recovery was more rapid following transfusion than without.

During the second crisis in this patient we were able to study the pigment metabolism and liver function. The marked sudden increase of urinary urobilin, rapid increase of bilirubinemia with change of the van den Bergh from indirect, through slight to moderate to strong direct type, the enlargement and tenderness of the liver, and the diminished hippuric acid synthesis as measured by the Quick intravenous test suggested diminished liver function. These findings then rapidly cleared with no therapeutic attempts. It can be seen from the pigment and hematologic studies that there was no marked increase of blood destruction associated with this series of events.

The study of hemoglobin metabolism in various blood dyscrasias has been given great stimulus by Watson's studies.[17,18] He reviews the question of the relationship of urobilinogen excretion to blood destruction and liver function, and concludes that alterations of blood destruction influence fecal excretion, but that change in liver function is the most important factor affecting urobilinogen excretion in the urine.

In our patient, there then followed a progressive rise of blood destruction of only moderate degree as measured by pigment excretion. Transfusions were begun to study their effect in inhibiting the hemolytic process. As can be seen from the chart, a preliminary decrease in blood destruction followed small transfusions (300 to 350 cc.) first of Type IV and then Type II. Following this a very marked increase in pigment excretion and a progressive drop in red blood cells, hemoglobin, and reticulocytes occurred. During the course of transfusions, the patient was continuously febrile. The spleen became progressively larger, the liver was tender and enlarged. The van den Bergh was slightly to moderately direct in type and there was continuous excretion of urobilin above the normal amounts (0 to 4 mg. normal). There was not the degree of alteration that was seen during the acute crisis from December 20 to 25, but qualitatively the changes were similar and appeared to differ only in degree. It appears that the retention of bilirubin in the body at this time might account for the apparent decrease of pigment excretion in the stools, but subsequently there was a marked increase of pigment in the stools and definite inhibition of blood regeneration. It is possible that sequestering and destruction of the transfused erythrocytes by the spleen and the infarctions accounted for the marked increase in the splenomegaly and of pigment excretion noted. Certainly the 1000 cc. of blood given over a

period of 12 days had little effect on the red blood cells and hemoglobin level of the peripheral blood. Suppression of bone marrow function is likewise evident from the drop in red blood cells, hemoglobin and reticulocytes.

Following splenectomy and massive transfusion, the red blood cells and hemoglobin remained at near normal levels. There was a sharp febrile reaction postoperatively and the plasma bilirubin was found elevated and showing a strong direct reaction. Meulengracht[12] has mentioned this sharp rise of temperature that occurs after splenectomy in many such patients, and Eppinger[16] has called attention to the change in the van den Bergh, which he ascribes to liver damage due to anesthesia. Possibly the livers of these patients are more susceptible to such nocuous influences as anesthetics of certain types.

The tenderness and enlargement of the liver disappeared rapidly and have not recurred in the year since splenectomy. The studies subsequent to splenectomy are illustrated in the chart.

Summary. 1. A case of congenital hemolytic jaundice is presented in which hematologic and pigment studies were carried out through the course of a crisis, repeated transfusions, and after splenectomy.

2. During the crisis certain observations indicated decrease of liver function which cleared spontaneously. There was no evidence of marked blood destruction. These changes have been observed also in certain crises of sickle cell anemia. The findings suggest the theory that liver disease may be a factor in the crises of such cases.

3. Transfusions were followed by a progressive enlargement of the spleen. There then occurred a marked increase in blood destruction, drop of red blood cells, hemoglobin, and reticulocytes. It is probable that the transfused red cells were sequestered in the spleen and rapidly destroyed, leading to the marked stercobilin excretion.

4. Splenectomy was followed by a return to normal in the red blood cells, hemoglobin, reticulocytes, bilirubinemia, and pigment excretion. All clinical symptoms and signs disappeared.

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THE HEART SIZE AND PULMONARY FINDINGS DURING ACUTE CORONARY THROMBOSIS

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Discussion in the literature of the incidence of cardiac enlargement and the degree of pulmonary congestion during and following coronary thrombosis has been based chiefly on the weight of the heart and the lung findings at necropsy. A few studies have been made of the effect of coronary thrombosis on the size of the heart as determined by Roentgen rays. But the infrequency of the Roentgen ray films taken in the various series have not permitted definite conclusion as to the presence or absence of cardiac enlargement in the days immediately following the coronary accident. A few case reports have been published concerning the development of left ventricular aneurysm following myocardial infarction.[3,7] Horine and Weiss[4] reported that 20 patients who had normal sized hearts at the time they experienced a coronary thrombosis did not develop roentgenologic evidence of cardiac enlargement although they were observed subsequently from 5 months to 9½ years. However, they reported only initial and final Roentgen rays, although in some cases *interim* Roentgen ray examinations were also made. Palmer,[5] in a study of the size of the heart after coronary thrombosis, found no example of acute dilatation of the heart in 27 patients examined within a month of the attack. He did find enlargement of the heart by Roentgen ray in 64% of 200 patients who had survived an attack of coronary thrombosis. About one-third of the patients failed to show or to develop enlargement though watched over periods averaging more than 3 years following the first attack of coronary thrombosis. Other authors[1,6] have expressed somewhat different opinions regarding size of the heart following coronary thrombosis. White[8] states that the enlargement, chiefly of dilatation of the left ventricle, may come on rapidly with cardiac infarction. Fishberg[2] states that the heart is not enlarged above the dimensions present prior to the occlusion. However, no report has been made in which a series of successive Roentgen rays of the heart were taken to discern the change in cardiac size which might occur in the days or weeks following the myocardial infarction.

Method of Study. The method of teleoroentgenography was used to determine the heart size and shape in the patients in this study. The Roentgen rays were taken with the patient in a sitting position. Great care was exercised in the handling of these patients because of the gravity of their cardiac condition. No untoward occurrences were encountered in any of the patients as a result of this study. The patients were permitted to sit up on the edge

of the bed without any active movement on their part, being given every necessary support by 2 individuals during the entire procedure. The Roentgen ray tube was placed 5 feet away from the patient and the film holder was kept in contact with the anterior surface of the chest. The Roentgen ray was taken during a phase of quiet respiration since marked changes can be produced in the size and shape of the heart by the diaphragmatic movements accompanying forced inspiration or expiration. The sitting position was chosen since the heart size may be increased in the recumbent position because of an increased return of blood to the heart in that position resulting in a physiologic dilatation.[9] Likewise the heart shadow may be decreased in the standing posture due to decrease in the return of blood to the heart. Furthermore, distortion of the heart size and shape by undue elevation of the diaphragm is less likely in the sitting than in the recumbent positions. In addition, standing would of course be contraindicated by the presence of a myocardial infarction.

The chief difficulty in the application of roentgenology lies, not in the technique, but in the interpretation of the normal limits of heart size, shape, and action, and, therefore, in the diagnosis of slight abnormalities. There are so many factors (*e. g.*, age, sex, habitus, respiration) resulting in individual variations within the normal range, that it is at present impossible to recognize them all, or to take them all into consideration in the evaluation of size or shape. Furthermore, important changes may occur in heart size or shape insufficient to produce definite roentgenologic abnormalities at the time of examination, but which would have been noted if comparative studies had been previously made. Obviously successive records of heart size and shape in the same individual carefully made should be more useful than a single comparison with a table of normal averages. It is, however, often impossible to possess any information about the Roentgen ray findings prior to the onset of trouble in a given patient. Certain measurements of heart size are in routine use and are of value. The one employed in this investigation is the cardiothoracic ratio which is the ratio of the transverse diameter of the heart shadow to the internal diameter of the chest. Although it admittedly is only fairly reliable as an index of cardiac enlargement, it is a simple and useful measure of heart size.

This study was made on 16 patients. Each had had attacks of undoubted acute myocardial infarction 12 to 60 hours prior to entering the Peter Bent Brigham Hospital. As soon after entry as possible (in the majority of cases within 24 hours of the acute episode) the initial Roentgen ray was taken in the manner already described. Subsequent Roentgen rays were taken with few unavoidable exceptions (2 patients died during the 2d week of hospitalization and contact with 2 patients was lost after the 3d and 4th months respectively) on the 3d, 5th, 12th and 28th days following admittance to the hospital and then at the end of the 3d and 6th months after the coronary accident. Figure 1 illustrates the successive Roentgen ray films of Case 13. In addition all but 3 of the patients had a roentgenkymogram taken usually on the 28th day of hospitalization. A total of 113 Roentgen rays was studied for this report. Furthermore, the patients were all followed carefully clinically with frequent examinations, electrocardiograms, blood pressure readings, blood counts and other pertinent data. The period of observation over which the patients were seen varied from 1 week to 7 months with an average for the entire group of 4.7 months. If the 2 deaths at the end of the first week were omitted, the observation ranged from 3 to 7 months with an average of 5.3 months.

The necessary measurements were determined from each Roentgen ray and the cardiothoracic ratio calculated. It was decided that a total change of less than 1 cm. in the transverse diameter of the heart could be considered to be within the limit of Roentgen ray error in this study, considering the difficulty in the technique in these very ill patients and the physiologic changes in heart size occurring among other things with the systolic and diastolic movements of the heart. It is true, however, that a change of less than 1 cm. when accompanied by a change of shape may have considerable significance, but a distinct change in shape did not occur in this series. In order to determine the presence of a significant change in the heart size in the successive Roentgen

rays of any one individual, the difference in the successive cardiothoracic ratios of each patient was calculated and then it was determined whether these differences were equivalent to a change in the transverse diameter of 1 cm. or more. Since Roentgen rays prior to the coronary occlusion were not available, the size of the heart on the successive Roentgen rays was referred to that on the first film. Special attention was paid to the appearance of the lung fields in the successive Roentgen rays to provide information upon the extent of pulmonary congestion in patients with acute coronary occlusion.

Results. The 16 patients studied present a number of clinical features, some of which will be analyzed in this report. Ten of the patients had had previous attacks of angina pectoris over periods varying from 3 weeks to 2 years, while 6 had no previous episodes of precordial or analogous pain. As is usually the case in this condition there was a great predominance of males, the ratio being 12 men to 4 women. The location of the lesion in the heart could be determined fairly accurately from the electrocardiogram since the electrocardiographic changes were quite marked in all the cases. Of the 16 cases, 12 had anterior myocardial infarctions and in the remainder the lesion was situated in the posterior wall of the heart. The blood pressures of 7 patients were moderately elevated during the first few days of hospitalization but all except 2 of these subsequently fell to more normal range. Of the entire series, 8 presented enlarged hearts initially, 5 were in the upper limits of normal and 3 were definitely normal. Six of the roentgenkymograms showed diminution or decrease of the normal pulsations along the left border, near the apex. The other 7 showed no abnormal change. Five complications occurred in the entire series. Two patients died from bronchopneumonia and perforation of the myocardium respectively; the other three complications included an attack of lobar pneumonia, phlebitis of the right and left lower extremities with pulmonary infarction, and a right hemiplegia subsequent to cerebral embolism. These 3 patients eventually recovered.

The lung findings in the Roentgen rays were sufficiently noteworthy to attract special attention. Only in 4 of the patients were the pulmonary fields essentially clear. The remainder showed either a fine or coarse diffuse mottling usually in the lower half of the lung fields and the hilar regions, or definite cloudiness in one or both of the lung bases. The pulmonary congestion, as a rule, was maximum in the first week and tended to decrease or disappear in the second week. The Roentgen ray photographs of Figure 2 demonstrate the initial evidence of pulmonary congestion and the subsequent clearing of the lungs during the first 2 weeks following the coronary accident. Auscultation of the lungs during the first week of hospitalization revealed the presence of basal râles in only 7 patients in spite of the fact that 12 of them showed roentgenologic evidence of pulmonary congestion. In the remaining 4 patients evidence of pulmonary involvement was lacking on both Roentgen ray and physical examination. It was found necessary to give digitalis to 3 patients who had somewhat more evidence of cardiac decompensation than the basal râles usually found in patients with coronary thrombosis. One of these patients had taken the drug before

the coronary occlusion and the other 2 required it on the 7th day following the accident because of increasing signs of cardiac failure.

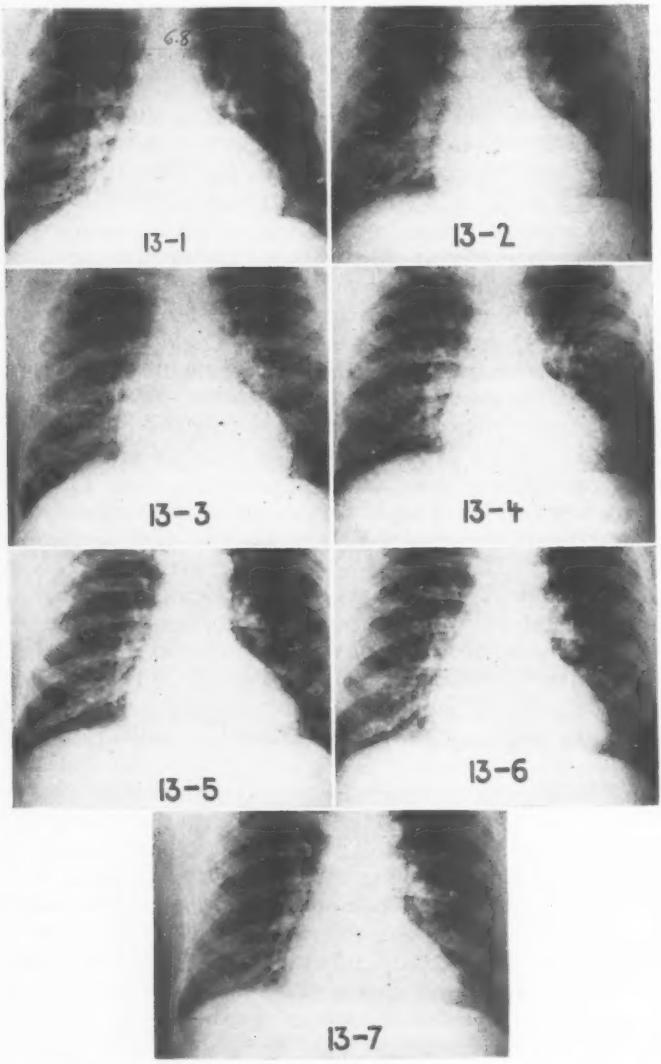

FIG. 1.—Seven successive Roentgen ray films of Case 13 taken over a period of 6 months following the coronary accident.

Eight patients showed no significant change in heart size in any of their films, while 8 others did show a significant increase or decrease in one or more of their Roentgen rays following the coronary occlusion. Table 1 shows the details of the changes in all the Roentgen rays of the series. Table 2 summarizes only those Roentgen rays which showed

a significant change (increase or decrease of 1 cm. or more) in the transverse cardiac diameter. In all the calculations, measurements taken on subsequent films were referred to those of the initial Roentgen ray which was used thus in the manner of a base line. The question arises as to what change, if any, occurred during the time elapsing between the onset of the cardiac accident and the time the first Roentgen ray was taken, but the data of this study do not give any information on this subject. It is noteworthy that 4 of the 8 patients included in Table 2 showed a significant change in only an isolated Roentgen ray and in 3 it was a decrease and in the other an increase. In con-

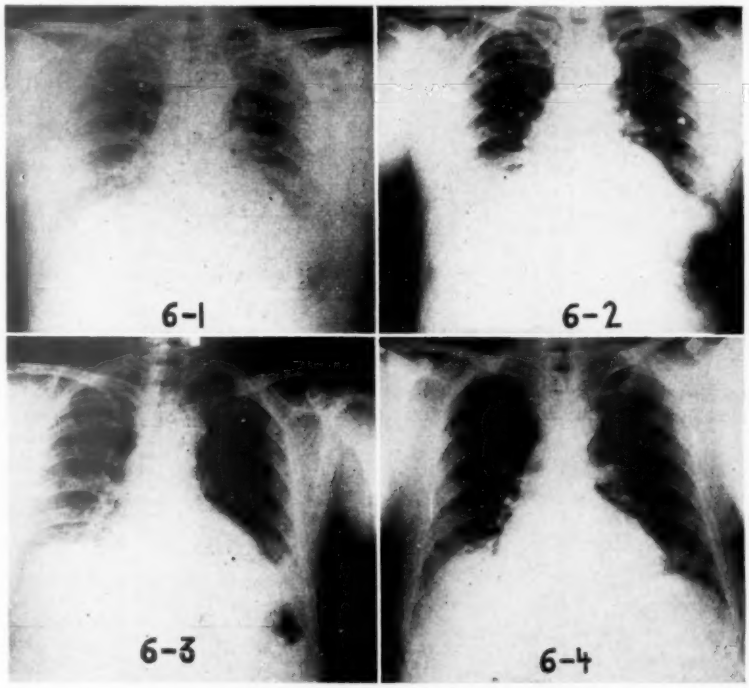

FIG. 2.—Roentgen ray films of Case 6 taken during the first 2 weeks following the coronary thrombosis. Note the progressive clearing of the pulmonary congestion.

trast, Case 16 presented a definite but temporary increase of 2.3 cm. on the 3d day and then reversion to the original size until the 4th month when the fifth Roentgen ray showed a decrease of 1.6 cm. Case 1 presented no change in heart size until after 1 month when the fifth Roentgen ray showed a decrease of 2.4 cm. and this was maintained to a lesser degree during the rest of the observation period. In only 2 patients was there a change on the second film which was subsequently maintained, and it is notable that in 1 of these patients (Case 3) it was an increase of 2 cm. which gradually progressed to 2.9 cm. at the end of 6 months. In contrast, in the other patient (Case 5), the

change was a decrease of 1 cm. which progressed to 2 cm. in 1½ months and later regressed to 1.2 cm. Summarizing these changes in cardiac measurements it is quite evident that there was no consistent difference in heart size in this study. Eight of the patients showed no impressive change in any of their entire series of films. Of the other 8 patients showing a significant change in cardiac size, 3 presented an increase in size in one or more films, and 5 a decrease in size in one or more films. In the important first 2 weeks following the coronary accident, only 4 cases had a change in cardiac measurements and in 2 they were increased while in the other 2 they were decreased.

TABLE 1.—CHANGE IN SIZE (IN CM.) OF CORRECTED TRANSVERSE CARDIAC DIAMETER COMPARED TO FIRST ROENTGEN RAY FILM

Case No.	Number of film					
	2d	3d	4th	5th	6th	7th
1	+0.2	0	−0.9	−2.4	−1.2	−1.0
2	−0.9	−0.3	−0.7	−1.4	0	−0.1
3	+2.0	+2.1	+0.9	+2.6	+2.7	+2.9
5	−1.0	−1.1	−1.0	−2.0	−1.0	−1.2
6	+0.7	+0.4	+0.1	−0.5	−1.2	−0.4
7	−1.7	−0.9				
8	+0.9	+0.5	+0.5	+0.4		
9	−0.3	+0.2	+0.1	+0.1	+0.2	+0.2
10	0	−0.5	+0.3	+0.2	0	−0.9
11	+0.1	+0.2	0	−0.7	−0.4	−0.3
12	−0.9	+0.6				
13	+0.1	−0.1	+0.3	+0.3	+0.8	+0.3
14	0	+0.1	−0.2	−0.1	+0.2	+0.1
15	+0.8	−0.6	0	+0.2	+0.6	
16	+2.3	−0.1	+0.7	..	−1.6	

TABLE 2.—CASES SHOWING SIGNIFICANT CHANGE IN HEART SIZE IN ONE OR MORE FILMS

Case No.	Time after attack Roentgen ray taken	Change in size (cm.) of corrected transverse cardiac diameter compared to first Roentgen ray film
1	1 mo.	−2.4
	3 mos.	−1.2
	6 mos.	−1.0
2	1 mo.	−1.4
3	3 days	+2.0
	5 days	+2.1
	1.5 mos.	+2.5
	3 mos.	+2.7
	6 mos.	+2.9
	3 mos.	+1.1
5	3 days	−1.0
	5 days	−1.1
	12 days	−1.0
	1.5 mos.	−2.0
	3 mos.	−1.0
	6 mos.	−1.2
6	3 mos.	−1.2
7	3 days	−1.7
16	3 days	+2.3
	4 mos.	−1.6

An attempt was made to see if there were any features about the patients which could be correlated with the presence or absence of an increase or decrease in heart size. It is interesting that all but 1 of the 8 cases with significant change included the first 7 patients studied in the series; this might tend to suggest the fact that the technique of

taking the Roentgen rays was improved as the study was continued. However, since no change was made in the technique it is much more likely that this occurrence is merely a coincidence. Table 3 compares the essential features of the cases which experienced a significant increase or decrease in cardiac measurements with those of the other patients. Considering the small number of cases in each series, however, it is impossible to state that the differences indicated in these tables point to any significant feature or features which characterize patients who show an increase or decrease in cardiac size following coronary thrombosis. Nevertheless, one point does attract attention and that is the occurrence of complications. Five complications were met with in the entire series and 4 of these, namely, lobar pneumonia (Case 3), hemiplegia with cerebral embolus (Case 4), bronchopneu-

TABLE 3.—CHANGES IN TRANSVERSE CARDIAC DIAMETER

		Patients with significant change	Patients without significant change
Sex	Males	7	5
	Females	1	3
Age	Range	40–63 yrs.	44–68 yrs.
	Average	51.8 yrs.	60.1 yrs.
Location of infarction (from electrocardiogram)	Anterior	5	7
	Posterior	3	1
Fever	Present	8	7
	Absent	0	1
Leukocytosis	Present	7	7
	Absent	1	1
Hours after attack 1st Roentgen ray taken	Range	12–60 hrs.	20–48 hrs.
	Average	35.5 hrs.	32.5 hrs.
Heart size on 1st Roentgen ray	Normal upper limit	1	2
	Normal	3	2
	Enlarged	4	4
Change in roentgenkymogram	Present	4	2
	Absent	3	4
Lungs	Râles on phys. exam.	2	5
	Cloudiness on Roentgen ray	7	5
Blood pressure	Elevated on admission	2	5
	Elevated subsequently	0	2
Digitalis administration	....	2	1
Complications	....	4	1
Period of observation	Range	1 wk.–7 mos.	1 wk.–6 mos.
	Average	5.1 mos.	4.5 mos.

monia followed by death after 1 week (Case 7), and pulmonary infarction with phlebitis in both lower extremities (Case 16), occurred in patients with change in heart size. Only one complication, death from perforation of the myocardium, appeared in the other group of cases but since exitus in this patient occurred at the end of the 1st week, it is possible that succeeding films might have shown change in the cardiac measurements. It is also interesting that 2 of the patients who received digitalis presented significant change in cardiac size, but in both cases digitalis was started on the 7th day following the coronary accident and there appeared to be no definite chronologic relationship between the beginning of digitalis administration and the occurrence of the change in measurements. The third patient to receive digitalis did not show any difference in cardiac measurements but in this case

digitalis had been given in 0.1 gm. doses before the accident and it was continued at the same schedule following it. Aside from the complications already mentioned, it did appear that the patients with a significant increase or decrease in heart size were somewhat more ill as a group than the others. Pain and fever lasted longer in these patients, blood pressure readings following the attack fell to somewhat lower levels in the neighborhood of 90 to 110 mm. systolic and in 1 case severe attacks of angina pectoris persisted although they had been present for only 3 weeks prior to the acute episode. The only audible friction rub present in the entire series also belonged to a member of this group.

Summary. 1. The change in heart size in 16 patients following unequivocal acute coronary thrombosis was studied by teleoroentgenograms taken over periods extending from 12 hours to 7 months following the acute attack.

2. No consistent change in cardiac size or shape was noted in this study. Eight of the patients showed no change in any of their entire series of films. Each of 4 other patients presented only one film with cardiac measurements significantly different from the others of their respective series and these were taken at greatly varying intervals (3 days to 3 months) after the attack with both increasing and decreasing measurements occurring. It is noteworthy that in the important first 2 weeks following the acute accident only 4 cases of the entire series had a change in cardiac measurements and in 2 they were increased while in the other 2 they were decreased.

3. It is impossible to state from this study that there is any significant feature which characterized the 8 patients who showed a change in cardiac size following coronary thrombosis. Nevertheless, the more frequent occurrence of complications within this group attracts attention. Aside from these complications it appeared that the patients with significant change in cardiac size were somewhat more ill than the others.

4. The findings in the Roentgen ray films of pulmonary congestion in the 1st and 2d weeks following the coronary accident were especially noteworthy. Twelve patients showed roentgenologic evidence of such pulmonary involvement, whereas in only 7 of these did auscultation reveal the presence of basal râles. In 4 patients evidence of pulmonary congestion was lacking on both Roentgen ray and physical examination.

The authors wish to express their appreciation to Dr. S. A. Levine and Dr. M. C. Sosman for their valuable advice and criticism.

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A CASE OF MYOCARDIAL INFARCTION MASKED BY BUNDLE BRANCH BLOCK BUT REVEALED BY OCCASIONAL PREMATURE VENTRICULAR BEATS

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The electrocardiographic signs of myocardial infarction are often obscured by intraventricular conduction disorders of high degree, especially by left bundle branch block.[2] In the following report a case is cited in which myocardial infarction associated with left bundle branch block was indicated in the electrocardiogram (E.C.G.) by ventricular premature beats, while the regular beats failed to display significant changes.

Case Report. E.S., a white male, aged 79, was admitted to the hospital on September 20, 1942. For 6 years he had suffered from pain in the chest on walking fast, especially on cold and windy days. The pain was relieved by nitroglycerine. Four days prior to admission the patient was seized by severe precordial pain and a choking sensation lasting about a half hour. Similar attacks came repeatedly and required the administration of morphine.

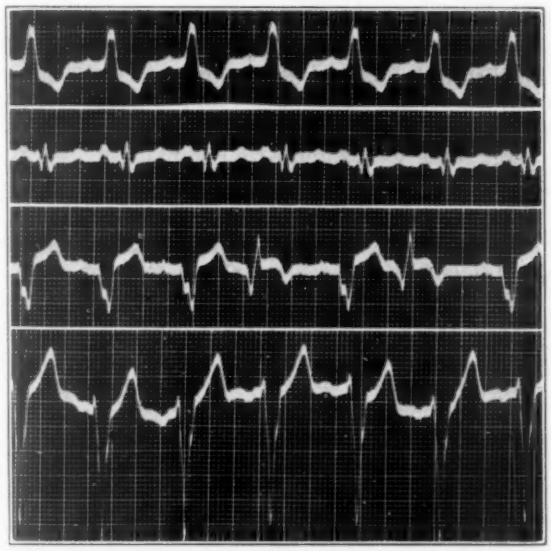

Fig. 1.—The complexes of the basic rhythm display the features of left bundle branch block. The 4th and 6th beats in Lead III are premature, followed by compensatory pauses; they have no P waves and are apparently of ventricular origin. Their shape is different from that of the regular beats, the broad Q wave preceding the R wave, an S–T segment is elevated and the T-wave inverted. These are the features usually displayed by normal beats in the presence of myocardial infarction of posterior site.

The temperature rose on the day of admission to 101° F.; the sedimentation rate (Westergren) was 39 mm. on September 24, 1942. E.C.G. examination

on September 22 and 28, 1942, revealed the signs of left bundle branch block but no significant changes suggestive of myocardial infarction. In a tracing taken October 2, 1942 (Fig. 1), 2 premature beats were observed in Lead III, apparently of ventricular origin. They had no P waves and were followed by compensatory pauses. Their shape differed from that of the regular beats, and were unusual for premature beats of ventricular origin. An R wave of moderate size was preceded by a broad Q wave of the same voltage. The duration of QRS was 0.12 sec. The S–T segment was elevated and the T wave inverted. Thus, the premature ventricular beats displayed features which are usually shown by regular beats in the presence of myocardial infarction of the posterior wall.

A diagnosis of coronary arteriosclerosis and infarction of the posterior surface of the left ventricle was made. The patient expired on October 5, 1942.

Postmortem examination revealed marked arteriosclerosis of the coronary arteries and recent thrombotic occlusion of the right circumflex branch about 4 cm. distant from its origin. There was an extensive recent infarction involving the posterior portion of the interventricular septum and the entire posterior wall of the right ventricle. In addition, numerous fibrous scars were found in the anterior and posterior walls of the left ventricle.

Excitations produced by a ventricular focus as a rule activate first the ventricle in which they originate and then spread through the interventricular septum to the other ventricle. Thus the contraction of the contralateral ventricle is delayed, as happens in bundle branch block. In fact the E.C.G. of premature ventricular beats resembles a great deal the tracing obtained in the presence of contralateral bundle branch block. However, there are exceptions. Occasionally, premature ventricular beats originate from a focus which is so located that the excitation wave may activate the two ventricles in approximately normal sequence. Then the tracing of the premature beats resembles normal complexes.[1] This may even happen when the regular beats display the features of bundle branch block.

In the presence of bundle branch block there is still another mechanism which may produce normal complexes in premature beats of ventricular origin. When, for instance, in the presence of left bundle branch block, premature beats originate in the left ventricle late in diastole at the time when the normal sinus excitation is due in the ventricles, the opposed effects of the dextrocardiogram (due to the normal excitation) and levocardiogram (due to the premature excitation) may combine to result in an approximately normal ventricular complex.[3] Such a mechanism was apparently not responsible in our case because the activation of the ventricles by the premature excitation was completed before the regular sinus excitation was due in the ventricles. The premature beats in our case were of the "intermediate type," that is, due to excitations which, because of the suitable site of the ventricular focus, activated the two ventricles in approximately normal sequence. Therefore the premature beats displayed the features of normal sino-auricular beats characteristically changed by the presence of myocardial infarction of posterior site.

Summary. A case is reported in which myocardial infarction was associated with left bundle branch block. Significant E.C.G. changes suggestive of myocardial infarction were absent in the regular beats but were displayed by premature beats of ventricular origin.

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SACCULAR ANEURYSM OF THE ABDOMINAL AORTA

Report of a Case With Terminal Anuria and Rupture into the Duodenum

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The infrequency with which one encounters saccular aneurysm of the abdominal aorta has been repeatedly emphasized. Kampmeier's[11] report (1936) of 73 cases from New Orleans brought the published cases to 386. In 1939 Lipschutz and Chodoff[13] reported 2 cases of their own and collected 41 additional cases from the literature. Since then Bigger[2] (1940) reported 2 cases in which surgical measures were employed, with success in one instance; Kommerell and Roemheld[12] reported 2 observed during 5 years in Berlin; Huberry and Pollack[8] collected 48 cases in the Cook County Hospital records from 1926 to 1940. Allen[1] (1940) described 2 producing urologic symptoms; in 1941 Jennings[10] added 4 cases and Uhle[15] cited 4 masquerading as primary genito-urinary disease; Eliason and McNamee[4] in 1942 reported 24 cases seen at Philadelphia General Hospital in a 10 year period. In addition there have been 4 single case reports comprising 90 cases since 1939 to which we are adding one described in detail. The addition of these 91 cases to the 429 listed by Lipschutz and Chodoff brings the present tabulation (up to December 1942) to 520 cases.

In the records of the Vanderbilt University Hospital since 1925 we have found no previous cases of saccular aneurysm of the abdominal aorta in 2796 necropsies and 22,040 medical admissions.

Case Report. A white salesman, aged 59, was admitted to the medical wards on September 25, 1942, complaining of left upper quadrant pain of 6 weeks duration. He had enjoyed good health until July 1, 1942, when he suddenly experienced chills, fever, and pleuritic pain in the right chest. He entered a hospital where a diagnosis of pneumonia was made. Recovery followed chemotherapy and he was discharged after 10 days. He then felt well until 6 weeks before his admission here when he noted the abrupt onset of pain in the left hypochondrium, which was sometimes sharp and at other times cramp-like, and usually radiated toward the navel. The pain was entirely unrelated to meals or bodily movements and was not relieved by soda. Although not progressive, it had been persistent and at times was severe, requiring codeine for relief. On a few occasions he had vomited small amounts of

undigested food, but there had been no hematemesis. There had also been no change in bowel habits and no history of tarry stools. In the 3 months preceding admission he had lost 35 pounds. He had had gonorrhea 20 years previously but syphilis was denied. The remainder of the history was noncontributory.

Physical Examination. The patient showed moderate weight loss and complained frequently of abdominal pain. His temperature was 99.6° F., pulse 80, respirations 30, and blood pressure 184/106 mm. Hg. The ocular fundi revealed evidence of arteriolosclerosis with slight arteriovenous compression. The chest was emphysematous but apparently clear. The heart was not enlarged to percussion, and there were no murmurs. The aortic second sound was accentuated. Pulsations of the peripheral vessels were equal and synchronous. There was diffuse abdominal tenderness and voluntary rigidity. The liver edge was barely palpable below the right costal margin on inspiration. No other viscera or masses were felt. There was no penile scar. The remainder of the physical examination was not remarkable.

Laboratory Examination. Hematologic studies revealed: 4.76 million red blood cells per c.mm.; hemoglobin, 13.9 gm. per 100 cc.; white cell count, 14,250 per c.mm. with a normal differential. The urine appeared clear with a specific gravity of 1.018, acid reaction, a 3+ albumin, and one granular cast as well as 3 white cells and an occasional red blood cell per high-power field. The stool was negative for occult blood, and no parasites were found. The non-protein nitrogen was 34 mg. per 100 cc. The blood Kahn was positive on two occasions and negative on a third; the Wassermann reaction was repeatedly negative.

Course in the Hospital. At first it was generally thought that this patient had a carcinoma of the stomach. Roentgenographic examination of the entire gastro-intestinal tract revealed no defect. The chest film was essentially negative. The electrocardiogram indicated a normal mechanism. His course remained unchanged for a few days, although frequent administrations of codeine were necessary to allay his pain. The albuminuria continued. Phenolsulfonphthalein excretion in 2 hours was 40%. Maximum concentration with the Fishberg test was 1.023. Urine culture was positive for *S. albus* and a non-hemolytic streptococcus, which were not considered significant.

On October 3, 1942 the abdominal pain became much more severe, and he had a chill with fever (103.8° F.). His white cell count was then 20,250. Chills and fever recurred on each of the following 2 days. He vomited frequently. No adequate explanation for these manifestations was apparent. A plain film of the abdomen on October 3 revealed no abnormality. On October 4 treatment with sulfathiazole was begun. The initial dose was 4 gm. with a maintenance dose of 1 gm. every 4 hours. The following day sulfadiazine was substituted because of increased nausea and vomiting.

On October 6 he did not void. With the thought that the anuria had resulted from chemotherapy, the drug was discontinued. The next day catheters passed to both renal pelves revealed no obstruction. Fifteen cc. of highly colored urine, obtained from the bladder, showed no sulfonamide crystals. There was no urine in the pelves, and water injected through the catheters returned clear. Parenteral fluids were forced, but they only produced peripheral edema. The non-protein nitrogen rose to 84 mg. per 100 cc. on October 7. On October 8 he was given 50 cc. of 50% dextrose intravenously, but the anuria persisted although the non-protein nitrogen had not increased significantly. On that day a lumbar puncture revealed clear spinal fluid under normal pressure with a negative Wassermann reaction.

At 1 A.M. on October 9, his 14th hospital day, he suddenly went into shock: He was semi-comatose, cold, perspiring profusely, had an ashen pallor, and his pulse was thready. Transfusion with 500 cc. of whole blood was followed by slight and temporary improvement. He then became progressively worse and died at 7.30 A.M. about 72 hours after the onset of anuria.

PATHOLOGIC FINDINGS (Dr. J. Harry Duncan): Saccular aneurysm of the abdominal aorta with thrombus formation. Compression of the left renal vein and inferior vena cava. Rupture into the third portion of the duodenum.

Gross Examination. There was about 150 cc. of blood-tinged fluid in the peritoneal cavity. The duodenum and jejunum were slightly distended, and there was a hemorrhagic discoloration with an extremely dark red line at their

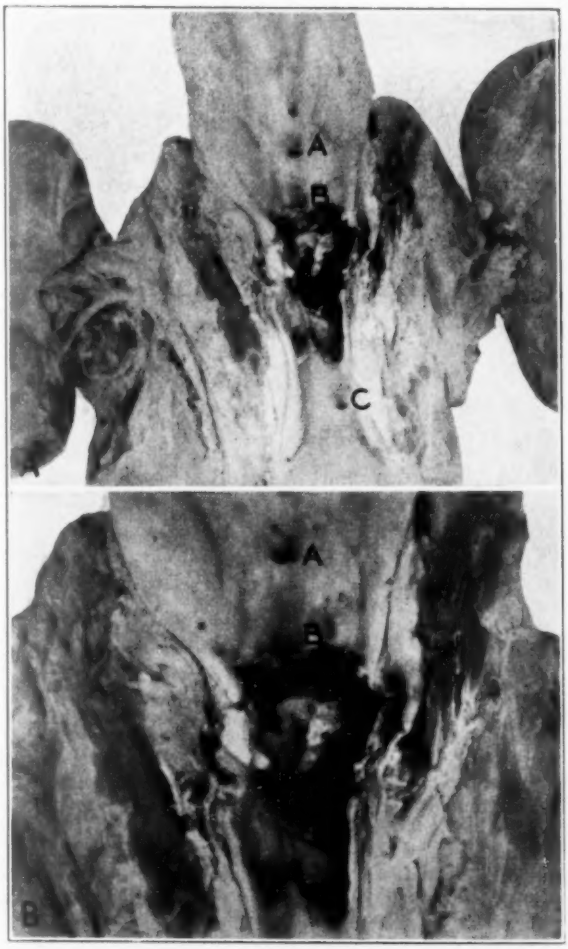

FIG. 1A.—Aorta opened posteriorly showing the aneurysm with thrombus *in situ* and orifices of (*A*) celiac axis, (*B*) superior mesenteric artery, and (*C*) inferior mesenteric artery. Orifices of renal arteries are concealed by the thrombus.

FIG. 1B.—Closer view of the aneurysm as pictured in Figure 1A.

junction with the mesentery. The ileum and large bowel did not show this change. Upon superficial examination the abdominal viscera appeared otherwise normal. The ascending portion of the aorta was slightly roughened and irregular and contained a few atheromatous plaques, but there was no associated longitudinal wrinkling or puckering.

The aorta was opened posteriorly revealing a large orifice, measuring 4 x

3 cm., in the anterior surface just below the celiac axis at the level of the superior mesenteric artery (Fig. 1). The edges of this opening were gray in color and very friable. Adjacent to this were numerous soft yellow plaques varying considerably in size and shape and containing several areas of ulceration. The entrance to the superior mesenteric artery was situated on the superior margin of this opening and was markedly distorted, presumably due to lateral dilatation of the aortic wall. The orifices of the renal arteries could not be found in the aorta. The foramen described above led into an irregular saccular dilatation measuring approximately 8 x 9 x 6 cm. In its right antero-lateral portion was a well-laminated pink-gray clot, firmly adherent to the wall and covering the orifice of the right renal artery, into which it extended for a distance of 1 to 2 cm. The orifice of the left renal artery was situated on the antero-superior margin of the sac wall and was substantially distorted and narrowed due to dilatation of the wall but contained no thrombus. There was a hemorrhagic nodular protuberance into the left side of the inferior vena cava, but the wall was intact. A perforation in the right antero-inferior margin of this sac communicated with a false pocket bounded posteriorly by the sac wall and antero-superiorly by the third portion of the duodenum where

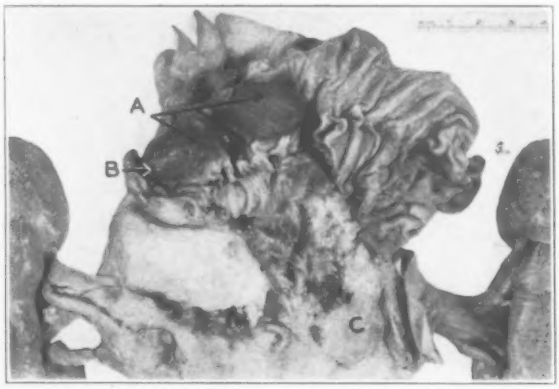

FIG. 2.—Anterior view of the aneurysmal sac and related structures. (*A*) Hematoma within posterior duodenal wall, (*B*) site of rupture, (*C*) anterior wall of aneurysm.

on the posterior wall there was a submucosal hematoma measuring 5 x 1.5 cm. (Fig. 2). The clot was firm, elevated, and well demarcated from the surrounding tissues and divided in its middle by a transverse groove. On the apex of the more distal elevation was a mucosal perforation 0.5 x 0.3 cm. There were no other areas of ulceration. The entire intestinal tract as far down as the sigmoid colon contained a cast of clotted blood with the marks of the intestinal folds imprinted upon it. The pancreas was rather firmly adherent about its head to the posterior wall of the aneurysmal sac but was otherwise not remarkable. Grossly the kidneys appeared normal. Both ureters were slightly dilated, but there were no mucosal lesions. The urinary bladder was not remarkable.

MICROSCOPIC EXAMINATION. (Since it was not possible to perform the necropsy until about 25 hours after death, there was a moderate amount of postmortem autolysis.)

Aorta. Sections taken at various levels, especially at the site of the aneurysm, revealed extensive atherosclerosis. The vasa vasorum exhibited marked hyaline thickening. Nowhere were there areas of necrosis in the media or perivascular accumulations of chronic inflammatory cells in the adventitia as evidence of a syphilitic aortitis.

Kidneys. The kidneys were edematous with considerable generalized tubular dilatation. There was no evidence of any significant pyelonephritis. Many of the glomeruli were filled with blood.

Prostate. Although gross examination of the prostate was not remarkable, a few small abscesses were seen microscopically.

FIG. 3

FIG. 4

FIG. 3.—Transverse section of the ascending aorta showing slight intimal thickening. Media and adventitia appear normal. *A*, Transverse section of vasa vasorum demonstrating atherosclerotic changes. × 22.

FIG. 4.—Longitudinal section through the aneurysmal wall revealing marked intimal thickening with overlying thrombus. The clear area (*A*) represents an arteriosclerotic plaque. Note dissection between intima and media (*B*) and within the media (*C*). Adventitia is a little thickened, and there are no perivascular cellular accumulations. × 22.

Discussion. Most of the clinical symptoms and signs can be reconciled with the pathologic findings. Because of the relationship of the aneurysm to the renal arteries, it seems evident that the albuminuria was the result of interference with the renal circulation, since "it is known from animal experiments how readily even transitory interference with either the arterial or the venous supply to the kidney produces albuminuria" (Fishberg).[5] The anuria was undoubtedly also due to impairment of the arterial blood supply to the kidneys because of obstruction of the right renal artery by the intra-aneurysmal thrombus and distortion of the orifice of the left renal artery. Since the kidneys had undergone very little pathologic change, it is assumed

that there was sufficient blood flow for their nutrition but not for the maintenance of glomerular filtration pressure. The gross dilatation of the ureters and the generalized tubular dilatation might represent a partial ureteral obstruction due to compression by the aneurysm. The bacteria isolated from the voided urine specimen presumably came from the prostatic urethra, in view of the abscesses noted in the prostate and the absence of any pathologic evidence of renal infection.

There seems to be no plausible explanation for the chills, fever, and leukocytosis which occurred on his 7th, 8th, and 9th hospital days. In a case described by Held and Goldbloom[6] the repetition of chills and high fever also constituted a puzzling feature. An explanation which they offered was that the aorta perforated so slowly as to give rise to an infectious periarteritis with a localized hematoma. In our case it seems conceivable that a slow leakage of blood from the aneurysm into the gastro-intestinal tract might have led to the febrile reaction.

The abdominal pain of which the patient frequently complained may have been the result of two factors: (1) stretching the aorta, causing stimulation of autonomic pain fibers; and (2) the distortion and consequent obstruction of the orifice of the superior mesenteric artery, which could produce the syndrome of mesenteric vascular thrombosis.[13]

It is obvious that this patient did not present all the classical symptoms and signs of saccular aneurysm of the abdominal aorta. There was no palpable, pulsating abdominal mass with an associated thrill and bruit. Such suggestive roentgenologic signs as erosion of the lumbar vertebræ, a soft tissue mass, calcification in the wall of the aneurysmal sac, and displacement of viscera were not evident. Examination of the lumbar spine at necropsy revealed no erosion of the vertebral bodies.

Although syphilis is believed to be the most frequent cause of aortic aneurysm, and especially of saccular aneurysm, it is conceded that in the older age groups arteriosclerotic changes play a significant rôle in the etiology. Despite the equivocal results with the Kahn test, the absence of evidence of syphilis in the pathologic findings indicates that the aneurysm in our case was the result of arteriosclerosis.

Aneurysms of the abdominal aorta most commonly rupture retroperitoneally. Rupture into the gastro-intestinal tract is a rare occurrence. Since these aneurysms occur most frequently in the upper segment near the celiac axis, the duodenum is the usual site of perforation. Kampmeier reported 10 cases of this type including one of his own. We have since been able to collect 4 additional cases from the literature. This report brings to 15 the total number of cases of aneurysm of the abdominal aorta with rupture into the gastro-intestinal tract.

That this condition may mimic primary urologic disease, such as perinephric abscess, nephrolithiasis, and pyelonephritis, has been shown by Allen, Duchanoff,[3] Rusche and Bacon,[14] and Uhle. Hoffman[7] has recently described a case wherein compression of the left renal artery by the aneurysm was associated with a hypertension which

he attributed to renal ischemia, comparable to that produced in Goldblatt's experimental animals. James[9] has reported a case of uremia associated with a saccular aneurysm of the abdominal aorta of 4 years duration. At autopsy there was marked bilateral renal atrophy apparently due to pressure on the renal arteries and ureters by the aneurysm, although this patient had not been anuric. Our search of the literature has disclosed no previous instance in which aneurysm of the abdominal aorta has resulted in anuria.

Summary. The recent literature relating to saccular aneurysm of the abdominal aorta (520 cases) has been reviewed with especial reference to cases with rupture into the intestinal tract (15 cases) and those associated with symptoms suggesting urologic disease.

A case, apparently of arteriosclerotic origin, is presented in which there was both anuria of 3 days duration and rupture of the aneurysm into the duodenum as a terminal event.

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SURVEY OF PROTOZOAN INFECTION OF THE STAFF OF A LARGE GENERAL HOSPITAL

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The results of surveys for the intestinal protozoan infections among hospital staffs have, as far as we know, never been published. Tsuchiya and Jean[1] (1940), in reporting the protozoan infection among freshman medical and dental students, mention that they made a survey of nurses, internes, attendants, and food handlers, but, at that time, published no results. For this reason, it was felt that a record of our survey on nurses, porters, members of the business office and others of the Graduate Hospital, University of Pennsylvania, might prove of reference value.

Material and Methods. Stool examinations were made on wet preparations, both in saline and in Lugol's iodine solutions, and on slides stained with Heidenhain's iron hematoxylin. At least 10 minutes each were spent in the examination of wet preparations and of the stained slides. Arrangements were made so that not more than 7 fresh specimens were submitted for examination in 1 day. Saline and Lugol stained smears were examined independently by both of us and the findings were combined to give the final total. For the most part, the stools had been passed less than 24 hours prior to examination. A freshly passed stool, however, is not necessarily a freshly formed stool, and trophozoites do not necessarily survive in constipated stools.

In computing our results, no infection was attributed to a particular species unless it could be verified on the stained slide. Exceptions to this were cysts of *Endamœba coli* and *Giardia*, which it was felt could be definitely determined in fresh material. Thus, although we found 136 protozoan infections, as a result of examination of both fresh and stained preparations, the species of only 114 of these could be positively identified.

Results. A total of 306 persons was examined. The following table records the incidence of infection among the group as a whole. The data are self-explanatory, and the incidence of infection is not unusual.

TABLE 1.—INCIDENCE OF INFECTION AMONG THE GROUP AS A WHOLE*

	No. of infections	Infection (%)
Total positive cases	136	44.4
Total identified cases	114	37.2
Endamœba coli	53	17.3
Endolimax nana	39	12.7
Giardia lamblia	31	10.1
Endamœba histolytica	11	3.6
Dientamœba fragilis	7	2.3
Iodamœba bütschlii	2	0.7
Chilomastix mesnili	2	0.7
Enteromonas hominis	1	0.3
Double infections	18	6.2
G. lamblia and *E. coli*	6	2.0
E. nana and *E. coli*	5	1.6
G. lamblia and *E. nana*	4	1.3
E. histolytica and *E. nana*	2	0.7
E. histolytica and *E. coli*	1	0.3
Triple infections	7	2.3
E. nana, *E. coli* and *D. fragilis*	3	1.0
E. nana, *E. coli* and *E. histolytica*	1	0.3
E. nana, *E. coli* and *C. mesnili*	1	0.3
E. nana, *E. histolytica* and *D. fragilis*	1	0.3
E. nana, *E. coli* and *G. lamblia*	1	0.3

* These numbers in each group represent only a portion of the persons employed by the hospital, since it was impossible to obtain the coöperation of the entire staff in submitting specimens. There was most difficulty with such groups as heads of departments, superintendents, internes and laboratory and social service personnel.

Each individual in the series submitted information concerning his extent of travel, past medical history, and present symptoms, if any. General good health was almost universally reported. Thus, the authors feel that symptoms may have been withheld, and that not much weight could be placed on the information presented. It was found that the extent of travel of the individuals examined had no effect on the incidence of infection.

An attempt was also made to determine the relative efficiency of wet preparations in contrast to stained slides. A comparison of the results obtained is of interest. A total of 93 infections were found on

the stained slides (M.L.), and 125 infections on fresh preparations. In the case of *E. coli*, where at times cysts may be scarce, an infection may be picked up in the fresh preparation which cannot later be found on stained slides. In our study, 23 infections of *E. coli* were found in wet preparations. On the other hand, *E. histolytica*, *Endolimax nana*, and *Dientamœba* (the last of which can be identified only with certainty as an "amœba trophozoite" in fresh preparations) were picked up several times on stained slides only; while 4 infections of *E. histolytica*, 5 of *Endolimax nana*, and 1 of *Dientamœba*. *Giardia* identification benefited from both methods; 3 were seen in wet preparations which were not found on the stained slides, while the stained method revealed 4 infections missed on the wet preparations.

Incidence of infection was recorded for the two principal race groups: negro and white. The incidence (Table 2) is somewhat higher for the whites, but not significantly so.

TABLE 2.—INCIDENCE OF INFECTION IN NEGROES AND WHITES

Race	No. of individuals examined	No. of positive cases	Infection %
Negro	93	31	33 3
White	213	83	38 3

Incidence of infection was also determined for the different occupational groups (Table 3). Most remarkable is the rather high incidence for the record room and business office.

TABLE 3.—INCIDENCE OF INFECTION IN OCCUPATIONAL GROUPS

	Nurses	Dietary dept.	Nurse's aid and maids	Porters, orderlies and elevator operators	Record room and business office
Individuals examined	81	61	55	47	40
Positive cases	30	15	20	15	23
% infection	37 0	24 6	36 3	31 9	57 5
Cases of *E. coli*	17	4	12	11	4
Cases of *E. nana*	9	7	7	2	9
Cases of *Giardia*	4	5	1	4	11
Cases of *E. histolytica*	4	1	1	2	2
Cases of *Dientamœba*	3	..	2	..	2
Cases of *Iodamœba*	..	..	1		
Cases of *Chilomastix*	..	..	1	..	1
Cases of *Enteromonas*	1				
Double infection	3	2	1	4	2
Triple infection	3	..	2	..	2

Summary. 1. Intestinal protozoan infection of 306 hospital staff members was found to be 44.4% (total identified protozoa, 37.2%; *E. coli*, 17.3%; *E. nana*, 12.7%; *Giardia*, 10%; *E. histolytica*, 3.6%; *Dientamœba*, 2.3%; *Iodamœba*, 0.7%; *Chilomastix*, 0.7%; *Enteromonas*, 0.3%).

2. Percentage infection was slightly (but not significantly) lower for negroes than for whites.

3. Incidence according to the occupations of the staff was higher, but not significantly so, in the record room and business office group.

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PERINEPHRIC ABSCESS—A PREVIOUSLY UNREPORTED COMPLICATION OF AMEBIASIS

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A search of the American, English and Spanish medical literature fails to discover a single instance of perinephric abscess complicating amebic dysentery. Furthermore, no author has suggested the possibility that *E. histolytica* may be the sole cause of such a complication. The case bears so many interesting points that it will be presented in detail, but an initial review of the literature on the complications of amebic infestation with special emphasis on urinary complications is desirable.

The most frequent intestinal complications are appendicitis, massive hemorrhage, perforation of the bowel with peritonitis, amebic granuloma, cicatricial stenosis of the bowel and pseudopolyposis. Among the extra-intestinal complications, which may occur in patients with or without symptoms of amebic dysentery are amebic hepatitis, perihepatitis, hepatic abscess, lung abscess, amebic bronchitis, amebic pleurisy, cerebral abscess, splenic abscess, cutaneous amebiasis, amebecemia, amebic pericarditis, amebiasis of the vagina, uterus, ovaries, Fallopian tubes, testicles, epididymis and penis. Amebic infestation of the gall bladder and biliary system has also been reported and subdiaphragmatic collections due to *E. histolytica* are not a rarity. A comprehensive analysis of the surgical aspects of the complications of amebiasis has been published by Ochsner and DeBakey.[6]

The urinary complications of amebiasis are cystitis, pyelitis, nephritis, urethritis and kidney abscess. In 1913, Külz[5] reported 2 cases of multiple kidney abscesses due to *E. histolytica*. Hartmann-Keppel[4] (1923) recorded a case of amebic kidney abscess following the operation of a hepatic abscess, and in 1924 Vichrew[8] reported one of amebic miliary abscess in the cortex of the kidney. The most illustrative of all cases of amebic abscess of the kidney was reported by Casco[2] in 1932. It related to a 4 year old boy who had suffered from relapses and exacerbations of bloody diarrhea for months, and whose feces were positive for *E. histolytica*. He developed a painful and tender enlargement of the left kidney and suffered from hematuria. Later, the patient passed large amounts of anchovy sauce pus per urethra. The kidney decreased in size and trophozoites and cysts of *E. histolytica* were found in the pus. He was treated with emetine quite successfully. Segurola,[7] in 1932, reported a case of amebic infection of the urinary

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bladder and of the kidney pelvis, without evident intestinal infestation and without a history of amebiasis, and presenting no symptoms referable to the gastro-intestinal or urinary tracts. Goyena,[3] in 1926, reported a case of intestinal and urinary amebiasis characterized by lumbar pain, albuminuria, pyuria and hematuria.

Bernardi and Esquivel[1] (1939) made an analysis of the literature on urinary amebiasis collecting over 50 cases and adding 4 of their own. In their report, it is evident that amebiasis of the urinary tract may present numerous symptoms, but pain in the lumbar region, tenesmus on urination, pyuria and hematuria are by far the most important. They believe that urinary amebiasis is always secondary to intestinal infestation by the parasite and that it is spread either through the blood or the lymphatics. They reviewed the case reported by Ingum (1914) of a man who was suffering from chronic gonorrhea and amebic dysentery and who used the same syringe for enemas as well as for urethral irrigations. This case is illustrative of another possible mode of infection, since the patient developed amebic cystitis. In their elaborate paper, no mention was made of a perinephritic abscess due to *E. histolytica*.

Case Report. L.G., a 47 year old Italian male, was admitted to the Philadelphia General Hospital on March 6, 1942, complaining of pain in the right side of the back and on the upper abdomen. Since 1933, he had suffered from relapses and exacerbations of a bloody diarrhea accompanied, on occasions, by severe crampy abdominal pains which, in many instances, simulated appendicitis. In October, 1941, his diarrhea recurred to a moderate degree with 4 to 7 bowel movements a day which on occasions consisted of only a small amount of mucus. In late November, 1941, he developed some fever and noticed an evanescent uncomfortable feeling in the right upper quadrant with slight tenderness on pressure. At this time, a proctoscopic examination in another hospital revealed a rectal mucosa which bled easily, but presented no ulcerations and a barium enema demonstrated irritability of the colon. In December, 1941, he was taken with severe constipation which persisted until time of admission, and his pain in the right upper quadrant increased in severity. No weight loss, but marked weakness was recorded. His social history revealed that at the time of onset of his illness in 1933, he was employed digging a well in southern New Jersey. No history pertaining to traveling was obtained.

On physical examination, at the time of admission, his temperature was 101° F., pulse 90, respirations 23, blood pressure 120 systolic, 70 diastolic. He appeared quite ill and showed marked pallor of the skin and mucous membranes. The costal margin flared more on the right than on the left, and the diaphragmatic excursions were diminished on the right side. Percussion note was flat at the right base up to the angle of the scapula with suppression of breath sounds in that area. Râles were heard on the left base posteriorly. There was a visible fulness in the right upper quadrant. The liver apparently extended 17 to 18 cm. below the 5th rib. It and the right costovertebral angle were exceedingly tender. Peristalsis was exaggerated and puffing and whistling in type. The rest of the physical examination was essentially negative.

Course. The temperature varied between 100° and 102° F. The blood picture on March 6, 1942 revealed a moderate secondary anemia and white cell count of 9600, of which 80% were neutrophiles, 15% lymphocytes, 2% monocytes and 3% eosinophiles. Five days after admission his blood picture was unchanged except for a left shift with a Schilling index of 1.9. The prothrombin time was 45% of normal. The stool examination was negative for *E. histolytica*. Roentgenographic studies revealed a slight elevation of the

right diaphragm with haziness at the base of the right lung. The liver was reported not enlarged.

Eight days after admission, laparotomy (Dr. E. L. Eliason) showed that the liver was diffusely enlarged, but no pus or intrahepatic or subdiaphragmatic masses were encountered. The gall bladder wall was thick and edematous, but no stones were palpable. The pancreas was normal. On the retroperitoneal surface, just below the liver, the tissues were dense, thickened and edematous and fluctuation was elicited. By blunt dissection carried around retroperi-

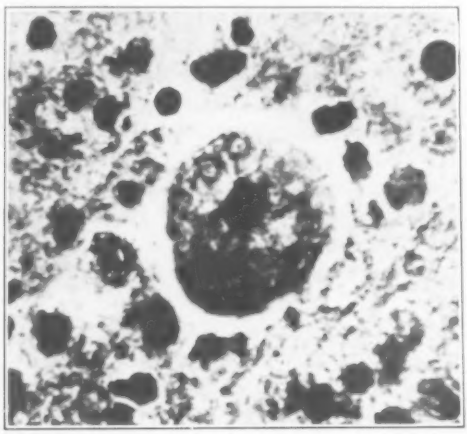

FIG. 1.—*Entamœba histolytica* trophozoite. Stained paraffin section of pus from perinephritic abscess showing two ingested erythrocytes. (× 1500.)

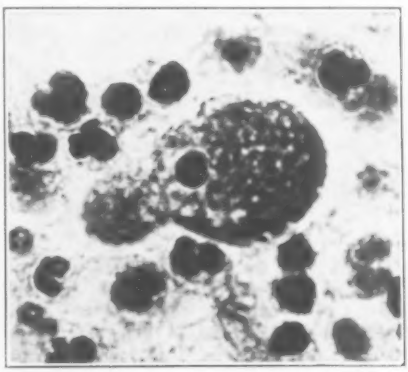

FIG. 2.—*Entamœba histolytica* trophozoite. Paraffin section of pus from perinephritic abscess. Note pseudopod. (× 1500.)

toneally to the perinephric space, a large collection of pus was opened and drained. The cavity occupied the entire perinephric space. The pus was thick and yellow, and was drained through a lateral stab-wound. Examination of the fresh pus failed to reveal any amebæ.

Postoperative Course. Considerable amounts of mucoid, blood-tinged, foul-smelling pus drained for a long time from the lateral portion of the incision. Cultures were positive for *Staph. aureus* and *Esch. coli*. Repeated examination of the pus failed to show *E. histolytica* cysts or trophozoites. Lipiodol visualiza-

tion of the abscess cavity localized it in the region of the 12th rib extending somewhat anteriorly and downwards to the level of the 2d lumbar vertebra. Intravenous urograms showed no abnormalities. Ten days after operation,

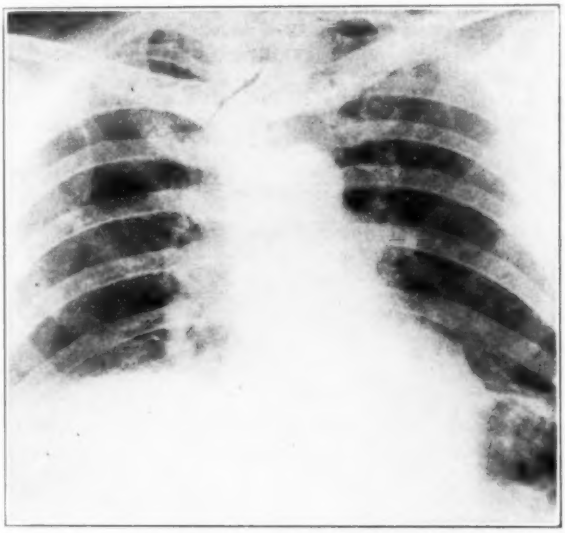

FIG. 3.—Preoperative chest film showing slight pleural reaction above and slight elevation of right diaphragm.

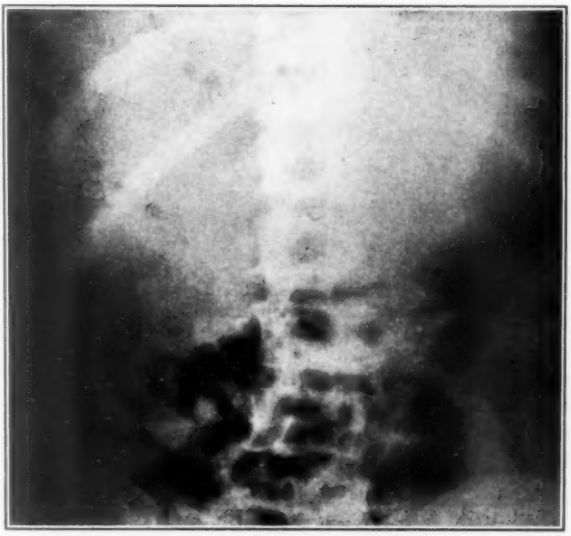

FIG. 4.—Preoperative flat plate of abdomen showing some hepatic enlargement.

blocked paraffin sections of the pus revealed *E. histolytica* in the vegetative forms and this finding was confirmed 3 days later. (This method is totally reliable, and the diagnosis of the condition cannot be questioned, in spite of the fact that the parasite was not found on fresh mounts.) (See Figs. 1 and 2.)

The temperature dropped to normal on the 3d postoperative day, flared up on the 23d and leveled off at 100° F. on the 36th day. Forty-six days after the operation, a course of emetine hydrochloride was started consisting of a hypodermic injection of 1 gr. of the drug daily for 12 days; this was repeated after a 2 weeks' rest. The abscess cavity was irrigated with emetine hydrochloride on several occasions. A course of vioform followed, and no amebæ were seen in the pus. No amebæ were found in the stools during or after completion of chemotherapy.

The patient improved remarkably fast after treatment, drainage ceased and he was discharged 131 days after admission and 123 days after operation.

FIG. 5.—Antero-posterior view of abdomen following lipiodol instillation into abscess cavity. The abscess is visualized laterally.

Comment. In the analysis of this case, it is evident that the patient suffered from a dysenteric form of amebiasis since 1933, as suggested by the relapses and exacerbations of bloody diarrhea; and that apparently there was involvement of the cecum and appendiceal regions by *E. histolytica*, since the patient had frequent attacks of pain in the right lower quadrant simulating appendicitis. Fever and dull pain in the right upper quadrant in November, 1941, obviates the supposition that he may have had a hepatitis secondary to the amebic colitis, or perhaps this was the initial symptom of the perinephritic collection of pus. The latter supposition is substantiated by the fact that the symptom picture in the right upper quadrant progressively increased

in severity. It can be assumed that the patient may have been suffering from the perinephric collection of pus since November, 1941. The first impression was that this patient was suffering from a subdiaphragmatic or hepatic abscess. There was, nevertheless, exquisite tenderness in the right costovertebral angle, which may have suggested kidney involvement. The blood picture of this patient did not suggest a purulent process, as there was a leukocytosis of only 9600. Examination of the liver and subdiaphragmatic space failed to reveal abscess formation. The thickness and swelling of the gall bladder wall is difficult to explain on the basis of an amebic infection. The dense wall and fibrosis of the perinephric abscess suggested a chronic course, but the character of the pus, on operation, was not typical of amebic infection. The failure to find the ameba in fresh mounts of the pus is not explained. The prolonged draining from the surgical incision may have been due to amebiasis of the tissues surrounding the wound, this being suggested by the rapid improvement after treatment with emetine hydrochloride.

As to the origin of the *E. histolytica*, we do not doubt that it was from the colon, but how it got to the perinephric space is difficult to state. Nevertheless, there are four possibilities for such a phenomenon. The patient may have had a hepatic abscess which ruptured into the perinephric space, but this is not evidenced by the findings on surgical exploration and it is a known fact that the majority of the hepatic abscesses due to *E. histolytica* rupture through the diaphragm into the pleural cavity. There is the possibility that this was a hematogenous metastasis, but we doubt it, since the parasite had to be filtered through the liver and lungs, and an abscess of the liver would have developed. The parasite may have been carried by the blood stream from an amebic infection of the lungs or bronchi, but this is hardly possible as there was no evidence of lung or bronchial involvement in the process, and this was a very strange localization for a blood-borne infection of this nature.

The last and strongest possibility is that the parasite may have passed through the lymphatics and localized in the perinephric space. The opinion is divided as to the lymphatic metastasis of amebic lesions, but Bernardi and Esquivel[1] are certain of its possibilities.

Summary. 1. A perinephric abscess—due to *E. histolytica*, apparently the first case—is reported. The diagnosis was proven by the demonstration of the parasite in the pus draining from the abscess after surgical intervention.

2. There is no doubt that there was involvement of the appendix and sigmoid, since the patient suffered from pain in the right lower quadrant suggesting appendicitis.

3. The complication was ushered in by a progressively severe pain in the right upper quadrant and pain in the right costovertebral angle.

4. The patient was explored and no evidence of a hepatic or a subdiaphragmatic abscess was found.

5. It is possible that the parasite traveled through the lymphatics and localized in the perinephric space.

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PROGRESS
OF
MEDICAL SCIENCE

PATHOLOGY AND BACTERIOLOGY

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GAS GANGRENE

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IN 1607 Fabricus Hildanus[36a] described a case of gas gangrene. Few clinical descriptions of the disease since that time have been clearer:

"On the 21st July, 1607, Estienne Topin wished, as work in the harvest reached its height, to lead himself an empty cart. He was at the time fifty years old, of robust build and in the best of health. As he was running he fell and the wheel of the cart tore up the whole of the inner part of the leg. The periosteum was ripped from the tibia for a palm's length; neither bone was, however, broken or cracked so that he was able to walk to his home about half an hour's walk away. . . . I was called the very same day: I anointed the whole of the thigh with oleum rosar and having washed the wound with red wine and tepid water . . . I enveloped the whole of the leg in a bandage soaked in vinegar . . . he had a restless night . . .

"On the 23rd . . . on removing the bandage it was seen that the part of the calf which had been separated from the underlying flesh required excision since it was turning livid and about to become gangrenous. I accordingly cut off this part . . . and having washed the stump with vinegar and salt I dressed it . . . and applied (a) poultice . . .

"On the morning of the 24th I undid the bandages and it was perfectly clear that the putrefaction had been arrested and that the gangrene was not spreading. . . . In the afternoon he was seized with an intense pain in the outer part of the leg . . . yet there was no outward sign and no obvious inflammation.

"On the 25th . . . I undid the dressings and found the external parts of the calf were black and mortified over a palm's width. . . . About midday . . . removing again the bandages I found the whole of the outer part of the leg and the foot itself mortified and covered with large black vesicles containing water similar to that in which meat had been washed: in certain places great pain persisted . . . Furthermore as I began to shave off some of the hair above the knee on the outer aspect at a certain place I could make out a sound as if there were some sort of empty space underneath. Inferring, therefore, that the disease was present underneath in that place I began to explain . . . that amputation was clearly going to prove fruitless . . . Two hours later . . . I saw that the place

about which we had heard the sound had become completely mortified. About ten that evening a vesicle the size of an egg arose in his groin beside the scrotum, black and filled with water like meat washings. On opening this the underlying flesh was black and gangrenous: within the next two hours the scrotum became swollen the size of a head and gangrenous. At about the third hour after midnight he became covered with sweat, at first hot then cold, and he died peacefully, in the middle of a sentence, four days, eleven hours after his illness commenced, and twenty-four hours after the onset of the gangrene."

Gas gangrene has generally, although not always, been a companion of wars. In the British armies in France in September 1914, between 10 and 12% of the wounded developed gas gangrene. Four years later the incidence had fallen to less than 1% of the wounded, as stated by the British Medical Research Committee Report on Anaërobic Infection.[56] In the French and German armies the incidence appears to have been slightly higher.[12,83] It was always found that the incidence was highest in periods of active operations when there was greatest delay in collection of the wounded. In civilian life the incidence is probably lower, though there are few data. Cramp (1912)[15] states that among 5802 accident cases in New York, 1909 to 1912, there were 9 cases of gas gangrene (0.16%).

1. The Etiology of Gas Gangrene. The relationship of the *Clostridia* to gas gangrene was long in confusion. Pasteur and Jobert (1877)[68] described *Vibrion septique* (*C. septicum*), and 4 years later Koch (1881)[40] described what he believed to be the same organism which was known in Germany as Koch's *Bazillus des malignen Oedeme*. Chauveau and Arloing's (1884)[13] study of gas gangrene appeared to indicate that it was a specific disease caused by this organism. But Welch and Nuttall (1892)[102] isolated *B. aerogenes capsulatus* (*C. welchii*) from gas gangrene cases and produced the disease in experimental animals. Within the next few years Welch's organism was rediscovered in both Germany and France. Fränkel in Germany called it *B. phlegmonis emphysematosæ*, and Viellon in France called it *B. perfringens*.

TABLE 1.—INCIDENCE OF VARIETIES OF GAS GANGRENE BACILLI

	France—War 1914–18				
	Weinberg and Séguin 91 cases, %	McIntosh 93 cases, %	Henry 50 cases, %	Germany peace-time 21 cases, %	Argentina 11 cases, %
C. welchii	71	55	80	100	46
C. novyi	34	4	10	33	10
C. septicum	13	16	16	5	27
C. sordellii	?	?	?	?	18
C. fallax	16	..	6	..	..
C. histolyticum	..	..	..	5	18
C. sporogenes	27	37	..	?	64

With the studies on the group carried on during the last war, order began to emerge. It became apparent that gas gangrene may be caused by one or a group of species of *Clostridia*. The distribution of species in gas gangrene infections is suggested in Table 1. The difference in the findings of different observers probably results from great diversity in occurrence of the species and in part it is due to different methods of isolation and identification used by the several observers. Moreover, these infections usually yield more than one species of *Clostridia*. In 37 of Weinberg and Séguin's (1918)[98] 91 cases of gas gangrene only 1 species of *Clostridia* was isolated; 29 *C. welchii*, 5 *C. novyi*, 1 *C. septicum*, 1 *C. fallax*, 1 *C. aerofœtidum*. In 54, approximately half the 91 cases, 2 or more species of *Clostridia*

were present and frequently also streptococcus, staphylococcus or other aërobic species.

2. Cultivation of Clostridia. The pathogenic *Clostridia* grow luxuriantly on a wide variety of culture media. Most species are able to utilize complex proteins or obtain their nitrogen from peptones, amino acids or simpler nitrogen compounds. Many species will utilize glycerol, starch or a variety of sugars. The older types of media were rich in proteins: chopped meat, liver or egg. The modern trend is toward media consisting of simple peptones—sugar solutions or synthetic mixtures.

The conspicuous feature of this group is the intolerance to oxygen. This necessitates cultivation either in an atmosphere with a low partial pressure of oxygen or in a medium poised at a negative oxidation-reduction potential. The degree of oxygen intolerance varies widely from species to species.

(*a*) *Surface Growth.* The most conspicuous landmark in the technique of the study of this group was the introduction of the McIntosh-Fildes anaërobic jar.[20a,21,53,59] Older procedures used for surface plating while generally successful gave variable results due largely to irregularities in oxygen pressure. If plates are inoculated from a wound exudate, for example, and incubated in an atmosphere of only moderately low oxygen tension, the more oxygen-tolerant species like *C. welchii* may grow while the oxygen intolerant species like *C. tetani* may fail to grow, thus leading to erroneous conclusions and frequently interfering with the isolation of pure cultures.

Many modifications have been made in the original Fildes' jar; Brewer's (1941)[8,9] jar is mechanically excellent, but the original principle remains unchanged. Two considerations are essential—the jar must be capable of holding a vacuum and means must be provided for keeping the platinized or palladinized asbestos catalyzing element dry and preferably heated when in action.

(*b*) *Growth in Fluid or Semisolid Media.* Cultivation of *Clostridia* in fluid media is much simpler than on the surface of solid media. The older unsatisfactory method of driving out dissolved oxygen by boiling and covering the surface of the medium with a layer of oil has largely given place to media poised at or near the optimum oxidation-reduction potential in which cultures are incubated exposed to air. This objective is achieved in chopped meat media,[42] or clear media may be adapted by the addition of suitable reducing agents and a trace of agar. The procedure of adding reducing substances has long been followed to a limited degree and many agents have been suggested.[1] The use of agar to retard oxygen diffusion has also frequently been suggested.[90b] Brewer (1940)[9] combined the use of highly efficient reducing agents, sodium thioglycolate or sodium formaldehyde sulfoxylate with a small concentration of agar. McClung (1940)[49b] and Reed and Orr (1941)[75c] have shown that all the known pathogenic species of *Clostridia* grow luxuriantly from small inocula in such media.

The optimum or limiting O/R potential for the various species has never been established, though Quastel and Stephenson (1926)[73] and Fildes (1927, 1929)[20b,c] have approached the points probably as closely as the complexity of the system will permit, and Reed and Orr (1943)[75g] have shown that all the known pathogenic species of *Clostridia* will grow from a minute inoculum in a simple peptone medium poised at an O/R potential level of Eh −100 to −200 volts.

Media prepared along these lines have given good results in biochemical reactions for the identification of *Clostridia*. (See Appendix.)

3. Proposed Procedure for Isolation of Clostridia From Wound Exudates. The following procedure has been in use in this laboratory for some years.[75c] It is in no way unique. It merely outlines a method which has generally given satisfactory results in a short period.

Swabs or fragments of tissue from an infected wound suspected of containing gas gangrene or tetanus organisms are smeared and stained by Gram's method* and are cultured as follows:

(*a*) Tubes of chopped meat medium are heavily inoculated. These are subsequently used only if the primary plating fails.

(*b*) Swabs or fragments of tissue are introduced into 8 ml. tubes of peptone-thioglycollate broth, well mixed, and 1 to 10, 1 to 100 and 1 to 1000 dilutions are made in similar broth. Generally less growth is obtained when saline solution is used for this purpose.

(*c*) From each dilution surface plates are then made on blood agar, clear peptone-thioglycolate agar and pour plates in semisolid thioglycolate agar. All are incubated in a Fildes type anaërobic jar. It frequently aids colony selection if a few plates are also incubated aërobically. The greatest difficulty in plating arises when spreading types are present. The worst offenders are of course *C. tetani* and species of *Proteus*, but *C. sporogenes*, *C. bifermentans* and even *C. septicum* at times are troublesome. This tendency is considerably reduced if the surface of the medium is kept relatively dry. Since moisture accumulates in the anaërobic jars, drying the plates before inoculation is of little aid. Better results are obtained by placing calcium chloride or "Dessigel S" in the anaërobic jars. We use a Petri dish cover about half filled with granular calcium chloride in the bottom of the jar and, in a deep jar with 12 to 14 plates, a second, similar dish of calcium chloride on the top of the pile of plates. The use of sodium azide as proposed by Lichstein and Snyder (1941)[43] to inhibit the growth of *Proteus* may be an advantage.

Subsurface growth in soft agar, as was shown by Greenberg (1941),[25] tends to be more discrete than surface growth on firm agar. If the inoculation is too heavy the growth may be diffuse, or, in the case of *C. tetani*, delicate irregular threads of growth may appear through the agar which are detectable only in a very clear medium with a good magnification. When the inoculum is light, even *C. tetani* and *Proteus* generally form discrete colonies.

Surface and subsurface colonies reach their maximum growth in 24 to 72 hours. If the media and the jars are satisfactory, colonies are distinguishable and may be fished at 18 to 24 hours.

Colony Form. Colonies of the members of the genus *Clostridium* have been described and figured by many authors, particularly Weinberg, Nativelle and Prévot (1937).[101] They may be considered as belonging to five fairly clearly defined groups, as follows:

Colony Form A: Large raised colonies, smooth to slightly folded, with entire or undulate margins, ordinarily reaching 2 to 4 mm. in diameter. The subsurface colonies are lenticular and entire or slightly irregular. Frequently smooth bulges appear on otherwise perfect lens-shaped colonies.

C. welchii is the most characteristic species of this group. Ordinarily the surface colonies are perfectly smooth and entire, though frequently the

* In using Gram's stain on members of this genus it is important, as shown by Spray (1936),[90b] to decolorize with alcohol and not with acetone or alcohol and acetone. The latter procedures give irregular results.

margins are slightly undulate. The subsurface colonies are perfectly lens shaped to slightly irregular, *C. butyricum*, *C. multifermentans*, *C. aerofætidum* and *C. paraputrificum* develop in similar colonies, but ordinarily the surface is slightly folded and the margins undulate.

COLONY FORM B: This type is represented by smaller, raised colonies, smooth to slightly folded or irregular, with entire to undulate or serrate margins, ordinarily reaching 1 to 2 mm. in diameter. The subsurface colonies are lenticular and are smooth to irregular, with short, thick rhizoids. This is the characteristic form of *C. tertium*, *C. carnis*, *C. sphenoides* and *C. difficile*.

COLONY FORM C.: This form is represented by one species in the group, *C. histolyticum*. The colonies are minute, raised, smooth and entire to slightly irregular with short rhizoids, ordinarily 0.1 to 0.2 mm. in diameter. The subsurface colonies are spherical to lenticular and entire to irregular, with short rhizoids.

COLONY FORM D: With this type there are moderate to large colonies, raised, irregular, with widespreading coarse rhizoids, 1 to 5 mm. in diameter. Subsurface colonies are occasionally spherical or lenticular, with widespreading rhizoids to irregular rhizoid masses. Colonies of the several species which grow in this form vary in respect to average size and extent of rhizoid spread: *C. cochlearium* and *C. tetanomorphum* colonies are small, 1 to 2 mm., with spreading fine rhizoids; *C. fallax* colonies are larger, 2 to 3 mm., with rather coarser rhizoids; *C. septicum* and *C. novyi* colonies are 2 to 4 mm., with rather coarse rhizoids.

C. sordellii and *C. bifermentans* tend to grow in still larger colonies with irregular margins and folded surfaces, generally without widespreading rhizoids, but some strains produce fimbriate colonies resembling those of *C. septicum*. Subsurface colonies are generally rectangular, frequently occurring in aggregates.

COLONY FORM E: This type is represented by *C. tetani*. Surface growth varies from irregular granular colonies with delicate spreading rhizoids to irregular spreading rhizoids without any definite formed colony structure. Subsurface colonies are irregular spherical masses with widespreading, very delicate rhizoids to single irregular rhizoid-like structures.

The usual colony form of the gas gangrene group is indicated in Table 2.

Surface growth on blood agar plates or subsurface growth in the 0.75% agar plates containing blood develop colony forms in general similar to those in clear peptone-thioglycolate media. For the most part, growth on or in blood agar is slightly more luxuriant, but the particular advantage is the hemolytic reaction. As is indicated in Table 2, all the toxin-producing species produce a zone of clear hemolysis about the colonies, somewhat more definite about subsurface colonies. The non-toxin-forming species do not produce hemolysis, with the exception of *C. sporogenes*, which ordinarily produces a narrow zone of partial hemolysis.

The most distinctive reaction occurs in the case of *C. welchii*. This organism ordinarily produces a double zone of hemolysis—an inner zone of complete clearing of the medium 5 to 10 mm. wide and an outer zone of partial hemolysis about 2 to 3 times as wide as the inner clear area. This double zone is apparent in a medium containing whole or defibrinated blood but not when preserved blood[74] or citrated blood is used.

It must be kept in mind that colony structure, especially when one is working from old laboratory strains, may be most deceptive. It was shown by Orr, Josephson, Baker and Reed (1933)[64] and by McGaughey (1933)[52] that *C. welchii* readily varies from S to R and that the R colony has more

the form of a *C. sporogenes* or *C. bifermentans* colony than the normal S form of *C. welchii*. Hoogerheide (1937)[34] found the same type of variation to occur with *C. histolyticum*, while Sordelli and Ferrari (1930)[89] and Spray (1933)[90a] briefly describe similar changes in several species in this group.

TABLE 2.—SURFACE COLONIES IN 24 HOUR CULTURES ON PEPTONE-THIOGLYCOLATE OR BLOOD AGAR AND SUBSURFACE COLONIES IN SEMISOLID AGAR; MORPHOLOGY OF ORGANISMS FROM THE SAME 24 HOUR CULTURES OR FROM PEPTONE-THIOGLYCOLATE BROTH

Species	Colony form (see text)	Hemolysis	Rods	Spores
Cl. welchii	A	+ double zone	Short, thick, capsulated	Very rare; oval, subterminal
Cl. butyricum	A	....	Short to long, thick	Oval, excentric to subterminal, swelling
Cl. multifermentans	A	....	Short to long, thick	Oval, central to excentric, swelling
Cl. aerofœtidum	A	....	Short	Very rare; oval, subterminal
Cl. tertium	B	....	Short to long, slender	Rare; oval, terminal, swelling
Cl. fallax	D	....	Short to long, thick	Very rare; oval, subterminal, swelling
Cl. paraputrificum	B	....	Short to long, thick	Rare, spherical, terminal, swelling
Cl. carnis	B	+ single zone	Short, thick	Oval to elongated, terminal
Cl. chauvœi	D	+ single zone	Short to long	Oval, excentric to subterminal, swelling
Cl. septicum	D	+ single zone	Short to long	Oval, excentric to subterminal, swelling
Cl. sphenoides	B	....	Short, oval to fusiform	Rare; spherical, terminal, swelling
Cl. novyi	D	+ single zone	Short to long	Large oval, subterminal, swelling
Cl. bifermentans	D	....	Short, thick	Oval, excentric to subterminal
Cl. sordellii	D	+ single zone	Short, thick	Oval, excentric to subterminal
Cl. sporogenes	D	± single zone	Short to long filaments	Oval, excentric to subterminal, swelling
Cl. histolyticum	C	+ single zone	Short to long	Oval, subterminal, swelling
Cl. tetanomorphum	D	± single zone	Short to long, slender	Spherical, terminal, swelling
Cl. difficile	B	+ single zone	Short to long, thick	Oval, elongated, subterminal, swelling
Cl. capitovalis	B to C	....	Short, slender	Oval, terminal, swelling
Cl. cochlearium	D	....	Long, very slender	Oval, terminal, swelling
Cl. tetani	E	+ single zone	Short to long, slender	Spherical, terminal, swelling

In our experience only S forms have been isolated from infected wounds—the aforementioned studies have shown that S to R variation is accompanied by a decrease in virulence. It is perfectly possible, however, that variant types may be found in infections.

(*d*) *Isolation.* In identifying organisms from the primary plates, single colonies are fished from the primary surface plates in the ordinary manner. A single large colony, such as that characteristic of *C. welchii*, provides ample material for the inoculation of the necessary diagnostic media. In the case of minute colonies like those of *C. histolyticum* it is an advantage to fish a single colony to a small deep tube of peptone-thioglycolate medium and incubate for a few hours.

In fishing minute subsurface colonies from a semisolid medium it is frequently an advantage to use a glass tube drawn out to a fine tip. After one has determined that the selected colony is free from spreading growth, which generally can be done with a 25 diameter binocular microscope and a subdued illumination from below the plate, the colony is stabbed with the capillary tube. The tube is then broken in the bottom of a peptone-thioglycolate broth tube.

The 10 tubes of diagnostic media, described in the following sections and listed at the top of Table 3, are then inoculated together with a plate of milk agar and preferably with additional plates to establish the purity of the cultures. Inoculation of a medium in a deep tube is most effectively done with a Pasteur pipette which delivers a few drops of inoculum at the bottom of the tube.

In many instances it will be possible to obtain pure cultures of *Clostridia* from the first plating of wound exudates, *i. e.*, in 24 hours. Frequently replating will be necessary.

4. Rapid Identification of Clostridia. As generally carried out the identification of species of *Clostridia* is a protracted process. We are of the opinion that, once a pure culture has been obtained, the newer types of cultivation permit as rapid identification as in most other groups of bacteria (Reed and Orr, 1941[7bc]).

The 5th edition of "Bergey's Manual" recognizes some 51 species belonging to the genus *Clostridium*. These may be roughly divided into four subgroups:

(1) Gas gangrene groups and related species, 20 species.
(2) Tetanus group, 1 specie.
(3) Botulinus group, 2 species.
(4) Non-pathogenic group, 28 species.

This article deals entirely with the first group.

A. *Biochemical Reactions.* Table 3 summarizes a series of biochemical reactions of most value in establishing identity of members of the gas gangrene group.

TABLE 3.—BIOCHEMICAL REACTIONS OF GAS GANGRENE CLOSTRIDIA

Species	Milk	Dextrose	Maltose	Lactose	Salicin	Sucrose	Hydrogen sulfide	Gelatin liquefaction	Nitrate reduction	Indol	Milk agar digestion	Exotoxin
Cl. welchii (perfringens)	Stormy	+	+	+	−	+	+	+	+	−	−	+
Cl. butyricum (group)	Stormy	+	+	+	+	+	+	+	+	−	−	−
Cl. multifermentans	Stormy	+	+	+	+	+	−	−	+	−	−	−
Cl. aerofœtidum	Stormy	+	+	+	+	−	+	+	+	−	−	−
Cl. tertium	Acid	+	+	+	+	+	+	−	+	−	−	−
Cl. fallax	Acid	+	+	+	+	+	+	−	+	−	−	+
Cl. paraputrificum	Acid	+	+	+	+	+	*	−	−	−	−	−
Cl. carnis	Acid	+	+	+	+	+	−	−	−	−	−	+
Cl. chauvæi	Acid	+	+	+	−	+	+	+	+	−	−	+
Cl. septicum	Acid	+	+	+	+	−	+	+	+	−	−	+
Cl. sphenoides	Acid	+	+	+	+	−	+	−	−	+	−	−
Cl. novyi (œdematiens)	Digested	+	+	−	−	−	+	+	−	−	+	+
Cl. bifermentans	Digested	+	+	−	−	−	+	+	−	+	+	−
Cl. sordellii	Digested	+	+	−	−	−	+	+	−	+	+	+
Cl. sporogenes	Digested	+	+	−	−	−	+	+	−	−	+	−
Cl. histolyticum	Digested	−	−	−	−	−	+	+	−	−	+	+
Cl. tetanomorphum	No change	+	+	−	−	−	+	−	−	+	−	−
Cl. difficile	No change	+	−	−	+	−	−	−	−	−	−	+
Cl. capitovalis	No change	+	−	−	−	−	*	−	−	+	−	−
Cl. cochlearium	No change	−	−	−	−	−	−	−	−	−	−	−
Cl. tetani	No change	−	−	−	−	−	+	*	−	−	−	+

1. MILK. The gas gangrene group may most readily be divided into two main divisions on the basis of reaction in milk, *i. e.*, those which produce "stormy fermentation" and those which do not. Milk containing reduced iron (see Appendix) permits this distinction to be made in 18 to

24 hours. As is indicated in Table 3, stormy fermentation is produced by *C. welchii*, *C. multifermentans*, *C. aerofœtidum* and *C. butyricum*.

Further separation on the basis of milk reactions is not so clear cut, but, as is indicated in Table 3, some 9 species form acid, which generally does not reach sufficient concentration in 24 hours to cause coagulation. Bromcresol purple shows an acid reaction. Three other species, *C. bifermentans*, *C. sordelli*, *C. sporogenes* and generally *C. noryi* and *C. histolyticum* give evidence of digestion in 24 hours, though in most instances the reaction is not complete before 48 hours or longer. The remaining four species, though they grow in milk, produce no reaction.

2. Sugar Fermentation. The most significant diagnostic differences in sugar fermentations are seen in the reactions to dextrose, maltose, lactose, salicin and sucrose. In the peptone-thioglycolate medium just described, if fermentation occurs, sufficient acid will have formed to give added bromthymol blue a frank yellow color in less than 24 hours, while sugar-free control cultures remain blue to the indicator. Since the indicator tends to be reduced by these organisms, it is necessary to add it at the end of the growth period and to read the reactions fairly promptly.

It is apparent from Table 3 that the stormy milk fermenters, with two exceptions, ferment all 5 sugars. Of those species which form acid in milk, the first 3 listed in the table, *C. tertium*, *C. fallax* and *C. paraputrificum*, ferment all 5 sugars. *C. carnis*, *C. chauvæi*, *C. septicum* and *C. sphenoides* ferment 4 of the 5, though they do not attack the same 4 sugars. Four species which digest milk ferment only dextrose and maltose, while *C. histolyticum* ferments no sugars. Of those which produce no change in milk, *C. tetanomorphum* and *C. difficile* ferment 2 sugars, while *C. cochlearium* and *C. tetani* ferment no sugars.

3. Hydrogen Sulfide. All species in this group produce some hydrogen sulfide when sulfur compounds are supplied in suitable form (Pacheco and Costa, 1940). The proteose peptone medium with bismuth or lead (see Appendix) as an indicator is definitely darkened or blackened in 24 hours by 17 of the 21 species in the group. *C. tetanomorphum* and *C. tetani* darken but do not generally blacken the medium in 24 hours, while *C. multifermentans*, *C. carnis*, *C. capitovalis* and *C. cochlearium* fail to cause any darkening of these media in 24 hours. The differentiation which Spray (1936)[90b] made between *C. tertium* and *C. fallax* on the amount of hydrogen sulfide produced is not apparent on these media.

4. Liquefaction of Gelatin. About half the species in this group produce rapid liquefaction of gelatin (see Appendix). The procedure has been to incubate the cultures at 37° C. and to test for liquefaction after the culture has stood for an hour in an ice-water bath.

5. Reduction of Nitrate. In the medium described (see Appendix) about one-third of the species in the group produce nitrites. The remaining species apparently fail to reduce the nitrate, as is shown by the fact that no nitrites are detectable at 24 hours. No distinction is made here between those species which fail to attack nitrate and those which break down nitrite as it is formed.[78]

6. Indol. In the tryptophane-containing medium described (see Appendix) about one-third of the species in the group produce indol, the remainder apparently failing to do so. No distinction is made here between those species which fail to break down the tryptophane and those which split the indol as it is formed.[78]

B. *Morphology*. The morphologic character of the organisms in this group as observed in 24 hour cultures on blood agar, peptone-thioglycolate

agar or peptone-thioglycolate broth is not distinctive. Sporulation, when present, is characteristic; but it is frequently absent even in cultures which sporulate freely 48 to 72 hours later. Smears from the young cultures, however, when well stained by Gram's stain without acetone, aid in checking the purity of cultures and frequently supplement the biochemical differentiation of species (Table 2).

A few cultures generally give atypical reactions: A few strains of *C. welchii* fail to give a positive nitrite reaction. As shown by Reed (1942)[78] this is due to the reduction of nitrites to ammonia.

In a few instances it has been impossible to distinguish between *C. septicum* and *C. chauvæi*.

A few *C. novyi* fail to ferment maltose in 24 hours.

A few *C. tetani* fail to produce hydrogen sulfide.

5. Toxins and Antigenic Structure of Clostridia. Pathogenicity of *Clostridia* is primarily associated with the elaboration of toxins. With certain important exceptions, the toxins are species specific. In sharp contrast most species exhibit a heterogeneous antigenic structure of the cells. This is made very clear in McCoy and McClung's (1938)[50] review. The heterogeneous character is evident in agglutination, precipitin and complement-fixation reaction. Little is known of the carbohydrate content of the cells. Meisel (1936)[57] isolated a polysaccharide from "*B. amylobacter*" and Jaminez (1931) and Reed and Orr (1940)[75a] have made similar isolations from *C. welchii* but the compounds proved to be nonspecific.

C. welchii Toxin. Bull and Pritchett (1917)[10] demonstrated soluble toxin in filtrates of *C. welchii* of human origin and from it prepared an antitoxin. This toxin they found contained two elements, a *hæmotoxin* and a lethal toxin which Henry called *myotoxin*. Much subsequent work appeared to indicate that all toxigenic strains of *C. welchii*, Henry (1920),[33] Reed, Orr and Campbell (1927), produced identical toxins as demonstrated by antitoxin neutralization.

Subsequently considerable controversy arose, which is still unsettled, concerning the relationship of *C. welchii* to flatulent diarrhea,[60] and toxemia of intestinal obstruction in man.[55] Also a group of organisms have been isolated from enterotoxemias, particularly of sheep, which closely resemble or are indistinguishable from the classical *C. welchii* of Welch and Nuttall except in the specificity of their toxins. The first of these was isolated by Gaiger and Dalling (1923),[23] Mason (1935),[48] sometimes called *Bacillus agni*. McEwen (1929)[51] then isolated a similar organism, *B. plaudis*, from *struck* disease of sheep. A third organism isolated from *pulpy kidney* of sheep by Bennetts (1932)[4] and later from other herbivorous animals has been called *B. ovitoxicus*. Wilsdon (1931)[103] classified these 4 organisms, on the basis of toxin neutralization, as *C. welchii* types A, B, C and D. The toxins and antitoxins display considerable overlapping of factors.[24,72] The present position may be summarized as follows:

Species	Type (Wilsdon)	Toxins produced (Glenny)
C. welchii (Welch and Nuttall)[102]	A	α, ζ
B. agni (Dalling)	B	α, β, ϵ, traces γ, ζ
B. paludis (McEwen)[51]	C	α, β, γ, traces δ
B. ovitoxicus (Bennetts)[4]	D	α, ϵ

The properties of these toxins in physiologic terms are: α, hemolytic, lethal, necrotic; β, lethal, necrotic; γ, lethal; δ, hemolytic; ϵ, lethal, necrotic; ζ, lethal.

So far only the A type, the classical *C. welchii* of Welch and Nuttall, has been isolated from gas gangrene of man or from the human intestine,[6,7] though as stressed by the latter authors, there appears to be ample opportunity for human infections with the animal types.

Early work on the production of *C. welchii* toxin[33,97a] involved cultivation in a complex meat infusion medium. MacFarlane and Knight (1941),[45] working with Type A, simplified the medium and greatly increased the yield of α toxin.

The older methods of assaying α toxin consisted in determinations of lethal effects by intravenous injection generally in mice, *in vitro* determination of hemolysis and necrotic effects by intradermal injection.[46] Nagler (1939)[59] observed a specific reaction between α toxin and normal human serum. When the two are mixed an opalescence occurs. MacFarlane *et al.* (1941)[46] have since shown a similar reaction to occur when toxin is mixed with the crude lecitho-vitellin solution obtained from egg yolk. Van Heynigen (1941)[96] has improved the quantitative features by estimating the toxin on the basis of the amount of turbidity produced and MacFarlane and Knight have shown a parallelism between toxicity, turbidity of egg yolk solution and lecithinase activity.

It remains to be demonstrated how satisfactory this α toxin produced on MacFarlane and Knight's type of medium and determined by lecithinase activity may be in the production of *C. welchii* toxin and antitoxin but it appears to have made possible the efficient production of these agents.

C. welchii Antigens. Little work has been done on the antigenic differentiation of A, B, C and D types beyond the specificity of toxins but among A types it has so far been impossible to establish antigenic relationship. Most reports based on agglutination reactions have indicated complete antigenic heterogenicity. Meisel (1936)[57] did find 6 of 10 Type A cultures belonged to one serologic group, but Reed and Orr (1940)[75a] found that of 85 Type A cultures, 6 showed close antigenic relationship on the basis of agglutination and precipitin reactions; a few others show overlapping of antigenic components and the balance show no antigenic relationship. Similarly Henderson (1940)[31d] found the O antigen of Type A to be strictly strain specific.

C. septicum Toxin. This species produces a monotypic toxin notwithstanding somatic antigenic differences, Robertson (1919–20),[80] Bengston (1933, 1934),[3] Pasternack and Bengston (1936)[67] have shown that the rapid killing on intravenous injection of the toxin is due to a specific toxin on heart muscle. Walbum and Reymann (1936)[97b,c] have obtained good yields of toxin on an infusion-peptone medium.

C. septicum Antigen. Felix and Robertson (1928)[19] indicated an H and O antigen in this species. They originally distinguished 4 types on the basis of H agglutinins and later Davesne (1928)[16] recognized 6 types. Bengston (1933),[3] however, could only identify 4 types. The several H antigenic types, Felix and Robertson (1928)[19] found to possess closely related O antigens and Henderson (1934, 1935)[32] found the O antigens of Types II and IV to be identical and distinguishable from those of Types III and I.

The O antigens of this species have a further interest. Robertson and Felix (1930)[81] claimed type specific protective value of O immune serum. Though questioned by Weinberg, Davesne and Haber (1932),[100] Robertson and Felix's[81] results have been confirmed by Henderson (1934, 1935).[31b,c] These considerations should be kept in mind in connection with the use of antitoxic sera.

C. novyi. Walbum and Reymann (1937)[97d] have obtained good yields of a specific toxin on an infusion-peptone medium.

It is impossible to come to any conclusion at this time concerning the identity of some 3 or 4 variously described "species," largely on account of conflicting reports of toxin specificity. *C. novyi* is generally regarded as synonymous with *C. œdematiens* of Weinberg and Séguin.[98] But Weinberg, Nativelle and Prévot (1939)[101] find antisera will neutralize toxin from all strains of *C. œdematiens* but not toxins of *C. novyi*. They also find that "*Bacillus bellonensis*" of Sacquépée (1916)[84] produces a specific toxin. They therefore consider the three to be valid species.

C. sordellii, C. bifermentans, C. œdematoides. There has been much difference of opinion as to the identity of these three problematical species. *C. sordellii*, described by Sordelli (1923)[88] and Hall and Scott (1931),[28] and *C. œdematoides*, described by Meleney *et al.* (1927),[58] were shown by Hall (1929)[26] to give cross-agglutination reactions as well as identical biochemical reactions. The latter author therefore suggested that they were identical and on the basis of priority proposed the name *C. sordellii*.

Later Clark and Hall (1937)[14] found cross-agglutination reactions with *C. bifermentans* and Stewart (1938)[92a] found *C. bifermentans* antiserum protected against *C. sordellii* toxin.

C. sporogenes. Hall and Stark (1923)[27] claimed some degree of homogenicity of all strains on the basis of agglutination, but Zeissler and Rossfield (1928)[105] recognized three groups. On the other hand, McClung (1937)[49a] and Smith (1937)[85] found some antigenic relationship with *C. parabotulinum* and *C. histolyticum*.

Other Species of Clostridium. Agglutination studies on several other pathogenic species have been of value in establishing identity: *C. paraputrificum* and *C. capitovalis*,[86a,87] *C. difficile*,[86b] *C. carnis* and *C. fallax*.[18]

6. Factors Which Influence Infection by Clostridia. The more important gas gangrene species are widely distributed in soils and animal feces. Zeissler and Neller (1927)[104] found the following distribution in 200 European soil samples: *C. welchii* present in 100%, *C. novyi* 45%, *C. septicum* 8%, *C. histolyticum* 2%.

This wide distribution, together with the fact that pathogenic *Clostridia* are frequently found in wound exudates where there is no evidence of *Clostridial* infection, raises the question of factors which permit or promote the development of infection. Fildes (1927, 1929)[20b,c] has shown that in the case of *C. tetani* spores will germinate in the tissues only when the surrounding fluid is poised at an Eh of +0.01 volts or more negative oxidation-reduction potential. Plotz and Geloso (1930)[70] extended these observations. We know that *C. welchii* and certain other species of the gas gangrene group will germinate and grow at a less negative O/R potential. However, this appears to be the most important factor in the development of *Clostridial* infections.

Several simple circumstances contribute to the production of favorable oxidation-reduction potentials in wounds. These may be summarized as: deep wounds in which air is excluded, the presence of devitalized tissue, toxic substances, foreign bodies, bacterial toxins. Dead or partially devitalized tissue, especially muscle, from which air is excluded, tends to assume a reducing potential. Certain substances, which may be introduced into a wound, as calcium salts, foreign bodies as cloth or soil, tend to promote necrosis and a reducing potential. It is obvious that a single spore or group of spores may be enclosed in microscopic masses of necrotic material which, with the exclusion of air or circulating blood, may become

sufficiently reducing to permit germination. Once germination and growth of a toxin forming species has occurred the accumulation of toxin will promote necrosis, reducing potentials, and thereby the spread of infection. On the other hand it has been shown by several authors,[11,17,95] that spores or even active vegetative organisms of several species of the gas gangrene group may be injected intramuscularly into animals without producing more than a slight transient reaction. Turner's results suggest, moreover, that spores which have been injected into tissues or introduced into wounds where they fail to germinate may develop at a considerably later data following injury or an unrelated infection which provides the necessary conditions for anaërobic growth.

In the production of experimental gas gangrene in guinea pigs, Reed and Orr (1942)[75b] found the following procedure the most efficient of several procedures tested: a wound was made deep into the thigh musculature, a fragment of excised muscle was placed in the lumen of the wound, 0.1 gm. of finely divided *sterile* soil and 1 drop of a 1 to 10,000 dilution of a young broth culture of the organism was added. The wound was then tightly closed with two rows of continuous sutures, one in the muscle, one in the skin.

Once spore germination has occurred and a local infection become established, extension of the infection is ordinarily rapid. It may remain localized in the wound, spread into a single muscle or group of muscles, spread into an entire segment of a limb or in the fulminating type there may be a general spread in all directions. The most important factors in the spread are fibrinolytic enzyme,[76] and toxin produced by the organisms.

Kropp and Smith (1941)[41] have shown that the earliest and most pronounced effect of the infection is to be seen in the connective tissue of the wound. Rapidly spreading edema appeared, involving first the subcutaneous connective tissue and a little later connective tissue of muscle bundles. Within 5 hours, in the case of *C. welchii* wound infections in guinea pigs, collagen fibers are swollen and fragmented and the cellular elements of the connective tissue largely broken down. This is to be seen not only where organisms were proliferating but beyond the margins where organisms are not detectable. At this stage and for some hours, connective tissue destruction with healthy skeletal muscle adjacent to it is the rule. There is no indication of muscle destruction in the absence of at least partial destruction of adjacent connective tissue. Gas spaces are early apparent as an accompaniment of edema. Blood-vessels of the connective tissue and muscle are thrombosed and frequently the endothelium of their walls rupture. Later destruction of muscle occurs and the infection appears to spread in the muscle. As the muscle is invaded, organisms make their way from the wound toward its extremities until it is totally destroyed. Frequently distinct zones appear. At the actual wound the muscle may be black, friable and difluent, succeeded by a deep red zone and sometimes a yellow band appears adjacent to a healthy portion. Gas can be demonstrated, first as bubbles between the muscle fibers and later in the surrounding areolar tissue.

Physical signs. These differ widely. In a strictly localized gas gangrene infection the only sign may be a foul-smelling discharge containing gas bubbles. As the infection spreads, swelling of the part appears and yields a tympanic note on percussion. At a further stage the swelling increases, the skin acquires a dusky hue and tympanitis and crepitation become more marked. Still later the skin shows mottling with purple patches and finally becomes greenish yellow. At times, however, the gangrene of deep muscle may be far advanced though covered by apparently normal skin.

Pathologic Anatomy. Details of the late pathologic changes are adequately described by Kettle (British Committee Report on Anaërobic Infection, 1919), Eliot (Medical Department of the U. S. Army in the World War, Vol. XI, 1927) and others.

Physical signs and gross pathology of infection by the different gas gangrene species of *Clostridium* do not differ very widely. In the case of infections with *C. septicum* or *C. novyi* there tends to be a more copious accumulation of mucoid material in the subcutaneous spaces and between muscles than in the case of *C. welchii* infections. In the former this material is more deeply blood stained and the skin ordinarily appears more deeply discolored. In the case of *C. sordellii* infections of animals the appearance is more like that of *C. septicum* or *C. novyi* than *C. welchii.* Where wound infections are complicated by the presence of proteolytic species, especially *C. sporogenes, C. paraputrificum* or *C. histolyticum* the exudates tend to be more foul-smelling and the local tissues of the infected wound tend to undergo slightly more rapid destruction.

7. Treatment of Gas Gangrene. (*a*) *Toxoids.* In a previous section, knowledge of toxin production by the principal gas gangrene species was reviewed. *C. welchii* toxoid has been prepared by Penfold and Tolhurst (1937, 1941),[69a,b] Plummer (1939)[71] and Stewart (1940, 1942).[92b,c] The latter author has shown that *C. welchii* toxins may be detoxified with 0.3% formalin, concentrated by ammonium sulfate precipitation and finally precipitated with alum. Guinea pigs with circulating antitoxin, as a result of immunization with this toxoid, are protected against many lethal doses of *C. welchii* toxin or culture.

C. novyi and *C. septicum* toxoids have been prepared by Weinberg and Barotte (1929)[99] and other work is now in progress in several laboratories on the more efficient production of these toxoids. As indicated in the earlier section on toxin production this will probably be successful.

It has been suggested that *C. welchii* toxoid, which is now available or could readily be made available in quantity, should be used in the army as a prophylactic measure. And, if and when *C. septicum* and *C. novyi* toxoids become available they should be combined with that of *C. welchii.* It is probable that these toxoids could be combined with T. A. B. vaccine—tetanus toxoid.

In these considerations for the future it must be remembered that gas gangrene is both a toxigenic and highly invasive infection. It is quite possible that active antitoxin immunity will prove to be inadequate.

(*b*) *Antitoxin.* Reports of the use of gas gangrene antitoxin either prophylactically or therapeutically are conflicting. The best of the last war reports are from Weinberg and Séguin (1918).[98] Of 50 wounded, treated with a polyvalent *C. welchii, C. septicum, C. novyi* serum 5 to 18 hours after injury, 25 died within a day without showing gas gangrene; the remainder recovered without developing gangrene. If these cases had followed the general average, 1 to 6 should have developed the disease. Of 30 patients with developed gas gangrene who were given polyvalent serum, 19 recovered. At the same time, of 66 gas gangrene patients who were given no serum 35 died. Newell (1939)[61] has advocated the early intravenous use of large doses of polyvalent antitoxin.

Gordon and McLeod (1941)[24a] have recently shown that specific antitoxic sera give good results in experimental *C. welchii, C. septicum* or *C. novyi* gas gangrene in guinea pigs.

The best method of preparing antiserum is still in question. Antitoxic serum prepared in horses by immunization first with formalized then with

pure toxin has given good results against experimental toxemia. But, as previously noted, Robertson and Felix (1930)[81] and Henderson (1934, 1935)[31b,c] have indicated that an antibacterial serum produced with the O antigen of *C. septicum* is superior to antitoxic serum in the prevention of this infection in experimental animals.

(*c*) *Roentgen Ray.* Kelly and Dowell (1936)[37] have advocated the use of Roentgen ray in treatment of gas gangrene. The results are far from convincing.

TABLE 4.—EFFECT OF SULFANILAMIDE AND TWO DERIVATIVES ON GUINEA PIGS INOCULATED WITH 10 MINIMUM LETHAL DOSES OF CL. WELCHII

Number of animals	Medication, gm.		Recovered, %	Died, %	Average survival time, hours
	Locally in wound	Orally			
		Sulfanilamide			
23	Nil	Nil	0	100	25
15	Nil	0.1, twice daily	0	100	33
20	0.15	Nil	40	60	87
30	0.15	0.1, twice daily	42	58	103
		Sulfathiazole			
25	Nil	Nil	0	100	26
5	Nil	0.1, twice daily	60	40	100
61	0.15	Nil	97	3	169
5	0.15	0.1, twice daily	60	40	170
		Sulfadiazine			
5	Nil	Nil	0	100	14
10	0.15	Nil	80	20	80

TABLE 5.—EFFECT OF SULFANILAMIDE AND TWO DERIVATIVES ON GUINEA PIGS INOCULATED WITH 10 MINIMUM LETHAL DOSES OF CL. SEPTICUM

Number of animals	Medication, gm.		Recovered, %	Died, %	Average survival time, hours
	Locally in wound	Orally			
		Sulfanilamide			
15	Nil	Nil	0	100	35
5	Nil	0.1, twice daily	0	100	72
8	0.15	Nil	25	75	84
5	0.15	0.1, twice daily	20	80	114
		Sulfathiazole			
15	Nil	Nil	0	100	37
5	Nil	0.1, twice daily	100	0	
15	0.15	Nil	87	13	222
5	0.15	0.1, twice daily	80	20	216
		Sulfadiazine			
5	Nil	Nil	0	100	31
10	0.15	Nil	100	0	

(*d*) *Chemotherapy.* Early work on chemotherapy of experimental gas gangrene in animals in which sulfonamides were administered orally gave indifferent results, Stephenson and Ross (1940).[91] Later experiments in which sulfonamides were given locally in the experimental infected wounds yielded highly successful results.[5,30,54,75b,d]

The results of one series of experiments[75d,e,f] are summarized in Tables 4 to 7. Results obtained by Hawking (1941),[30] McIntosh and Selbie (1941)[54] and Bliss, Long and Smith (1942)[5] are, in general terms, similar. These data are based on experiments in guinea pigs in which experimental wound infections were established by a method described in Section 6 of this paper. Where oral treatment was used the first dose was given 1 hour previous to wounding and continued, 2 doses per day, 0.1 gm. per dose in 300 gm. guinea pigs. In local treatment the drug, as a dry powder, was introduced at the time of infecting, a single dose of 0.15 gm. for 300 gm. guinea pigs, or at various intervals after infection.

In general it will be observed from Tables 4 to 7 that oral treatment

was definitely inferior to local wound treatment, although with the more efficient drugs, sulfathiazole and sulfadiazine, the contrast between local and oral treatment was not as great as with the less efficient sulfonamides.

As indicated in the tables, sulfanilamide was least efficient and sulfathiazole and sulfadiazine most efficient of those tested. Sulfapyridine, sulfamethylthiazole and sulfanilylguanidine (not shown in the tables) were intermediate in their action between sulfanilamide and sulfathiazole or sulfadiazine.

TABLE 6.—EFFECT OF SULFANILAMIDE AND TWO DERIVATIVES ON GUINEA PIGS INOCULATED WITH 10 MINIMUM LETHAL DOSES OF C. NOVYI

Number of animals	Medication, gm.		Recovered, %	Died, %	Average survival time, hours
	Locally in wound	Orally			
		Sulfanilamide			
15	Nil	Nil	0	100	35
18	Nil	0.1, twice daily	22	78	55
25	0.15	Nil	24	76	69
23	0.15	0.1, twice daily	22	78	97
		Sulfathiazole			
8	Nil	Nil	0	100	46
5	Nil	0.1, twice daily	100	0	
20	0.15	Nil	85	15	109
5	0.15	0.1, twice daily	80	20	162
		Sulfadiazine			
5	Nil	Nil	0	100	31
10	0.15	Nil	40	60	121

TABLE 7.—EFFECT OF SULFANILAMIDE AND TWO DERIVATIVES ON GUINEA PIGS INOCULATED WITH 10 MINIMUM LETHAL DOSES OF CL. SORDELLII

Number of animals	Medication, gm.		Recovered, %	Died, %	Average survival time, hours
	Locally in wound	Orally			
		Sulfanilamide			
11	Nil	Nil	0	100	32
5	Nil	0.1, twice daily	0	100	52
3	0.15	Nil	66	34	56
5	0.15	0.1, twice daily	40	60	82
		Sulfathiazole			
5	Nil	Nil	0	100	24
17	0.15	Nil	0	100	62
		Sulfadiazine			
5	Nil	Nil	0	100	21
10	0.15	Nil	0	100	65

Of the four most important gas gangrene species of *Clostridia* tested, it is apparent from the tables, *C. welchii* infections were most readily controlled; *C. septicum* and *C. novyi* less responsive but well controlled by sulfathiazole. *C. sordellii* infections were not significantly influenced by any chemotherapy tested. It has been previously noted, however, that *C. sordellii* is a relatively rare organism. In mixed infections or where one or more of *C. welchii*, *C. septicum* or *C. novyi* was introduced along with *C. sporogenes* or *C. histolyticum*, chemotherapy was as effective as against a single infecting organism.

When gas gangrene infections were permitted to become well established in the animals before chemotherapy was started, results were inferior or negative. In experimental animals where fatal infections developed in an average of 25 to 35 hours, a delay of 3 hours in initiating local treatment had no significant influence on the effects of chemotherapy. A delay of 5 to 6 hours, when infection was established and extended well beyond the local site of the wound, chemotherapy proved to be only slightly effective. Longer delay rendered the chemotherapy useless.

The advantage of local over oral administration appears to be largely a matter of local concentration in the infected or potentially infected tissues. Reed and Orr (1941)[75b,c] have shown (Fig. 1) that when sulfanilamide or sulfathiazole are introduced into experimental wounds the concentration of drug in the musculature of the wounded leg is much higher than in the blood stream. In contrast when the drug is administered orally the concentration is the same in all tissues as is to be expected from the results of Marshall, Emerson and Cutting (1937).[47]

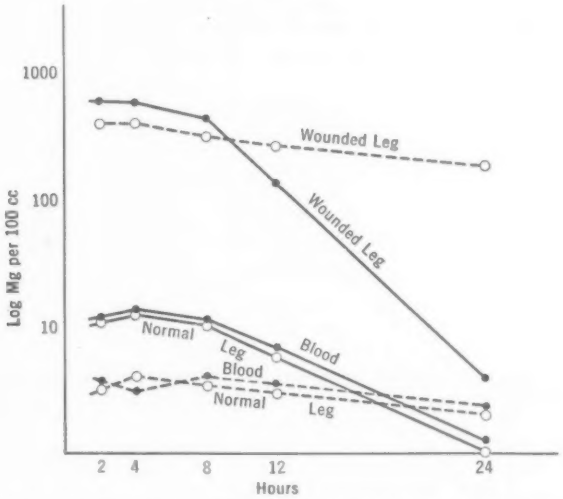

FIG. 1.—Changes in concentration of sulfanilamide (solid lines) and sulfathiazole (broken lines) in blood, normal muscle of leg and wounded muscle of leg following a single 0.15 gm. dose applied locally in the wound.

The large concentration of sulfonamides in wounds does not appear to impede healing.[36] Reed, Orr and Anderson (1942)[77] have shown that sulfathiazole up to 80 mg. per 100 cc. in serum does not retard the growth of fibroblasts in tissue cultures. In higher concentrations growth of fibroblasts is inhibited but if after several days' exposure to saturated solutions of sulfathiazole the cells are transferred to normal serum characteristic growth occurs. Reed and Orr (1942)[75e] have also shown that polymorphonuclear leukocytes are able to phagocytose staphylococcus at a normal rate in the presence of concentrations of sulfathiazole up to 80 mg. per 100 cc. Kropp and Smith (1941)[41] found that in experimental gangrene in guinea pigs treated locally with 0.15 gm. of sulfathiazole there is a massing of leukocytes and small lymphocyte-like cells at the periphery of the wound or a few millimeters beyond it which began to appear a few hours after infection. This condition was absent in untreated animals. Macey (1941)[44] and Klepser and Veal (1942)[38] state that human skin grafts develop normally in the presence of large concentrations of sulfanilamide and some of its derivatives.

In applying these animal results to man it cannot be emphasized too strongly that they indicate chemotherapy to be a prophylactic and not a definitive treatment of gas gangrene. Sulfonamides should be applied as soon as possible after a wound has been inflicted, with the objective of

retarding a possible infection until circumstances permit proper surgical procedure. It is impossible to suggest time limits, as no two wounds are alike or receive similar inoculations, but it is evident that the drug must be introduced before there has been any extensive gas gangrene infection—this may be a matter of a few hours or it may be safely delayed for several hours.

Surgical treatment should be introduced regardless of how early the sulfonamide may have been introduced. As Fletcher and Raven (1940)[22] and Ogilvie (1940)[62] suggest, the surgery will depend upon the condition of the patient. The procedures include: complete excision of the wound with removal of foreign and injured or infected tissue. All muscle tissue showing departure from the normal should be excised. To accomplish this it may be necessary to excise a complete muscle from origin to insertion, or even to sacrifice a group of muscles. In the case of fulminating infection, amputation may be necessary. If chemotherapy is applied early and the drug well distributed in or over the wound area extensive infections should be rare.

When débridement is complete *sterile* sulfonamide may again be applied. Present knowledge suggests that sulfathiazole or sulfadiazine are the most valuable.

Many surgeons at the present time supplement local chemotherapy and débridement with the Orr-Trueta immobilization procedure.[94] The early Trueta procedure of immobilization without chemotherapy as a means of preventing the development of gas gangrene has been questioned by many bacteriologists. In experimentally infected animals it has proven to be a failue.[75f]

(*e*) *Combined Chemotherapy and Serum Treatment.* There is nothing to contraindicate the combination of these two forms of therapy. Henderson and Gorer (1940)[32] have shown that in the case of *C. septicum* in experimental animals, sulfapyridine and antibacterial serum are more effective than either drug or serum alone. Reed and Orr (1942)[75d] have shown, in a small group of animals infected with *C. sordellii* that a combination of sulfathiazole and specific antitoxin resulted in recovery, whereas either treatment alone had no significant influence on the infection.

APPENDIX

Media for Clostridia

The following media were suggested by Reed and Orr (1941)[75c] for the isolation and identification of gas gangrene *Clostridia*.

1. *Plating Media, Surface Colonies.* (*a*) Blood agar is satisfactory for most purposes and has the added advantage of differential hemolytic reactions. (*b*) Clear plating media has certain advantages in the differentiation of colony types. Two formulæ have given equally good results:

A. Brewer's sugar-free broth, desiccated	62.5 gm.
Dextrose	1 gm.
Agar	20 gm.
Water	1000 ml.
Adjust to pH 7.6	
B. Proteose peptone	20 gm.
Na_2HPO_4	2 gm.
Dextrose	1 gm.
Sodium thioglycolate	1 gm.
Agar	20 gm.
Water	1000 ml.
Adjust to pH 7.6	

2. *Plating Media, Subsurface Colonies.* For subsurface colonies either *A* or *B*, above, with 7.5 gm. of agar to the liter have been satisfactory. This is the procedure of Greenberg (1941)[25] except that he used test tubes, whereas we prefer to use plates in anaërobic jars.

3. *Fluid Media for Biochemical Reactions.* (*a*) *Milk:* Of several preparations tested, the most satisfactory has resulted from following the suggestion of Hastings and McCoy (1932)[29] to add "reduced iron." More uniform results have been obtained with dried skim milk than with fresh milk. Bacto dried milk and a commercial Canadian product, milkite, have proved equally suitable. One hundred grams of dry milk is mixed with 1000 cc. of cold water, strained through gauze, adjusted to pH 6.8 (more alkaline milk darkens on autoclaving) and dispensed to tubes to which 0.05 to 0.1 gm. of reduced iron (Merck's "reduced with hydrogen") had previously been added. In this medium 5 to 100 organisms provides a suitable inoculum. Stormy fermentation, when it occurs, is generally marked in less than 24 hours.

(*b*) *Sugar-free base for fermentations:*

Bactopeptone or proteose peptone	20 gm.
Sodium chloride	5 gm.
Sodium thioglycolate	1 gm.
Agar	1 gm.
Water	1000 cc.

For fermentation reactions the sugars should be sterilized by filtration and added to the sugar-free base after autoclaving. Bromthymol blue is a satisfactory indicator, but since it tends to be reduced during the growth of anaërobes it is added at the end of the growth period and the reactions are read at once. Cultures in the sugar-free base or with a non-fermentable sugar present remain on the alkaline side of this indicator. Fermentable sugars, without exception, are sufficiently broken down in less than 24 hours to give a frank acid reaction. Production of gas has not been considered, as some species produce it from peptones.

The most significant differences between the 20 odd species of gas gangrene bacilli are to be seen in their action on dextrose, lactose, maltose, salicin and sucrose.

(*c*) *Liquefaction of gelatin:* The best results have been obtained with the following formula:

Gelatin	50 gm.
Bactopeptone	10 gm.
Sodium phosphate (Na_2HPO_4)	2 gm.
Dextrose	1 gm.
Sodium thioglycolate	1 gm.
Water	1000 cc.

All species in the group grow rapidly in this medium. Those which liquefy gelatin within 10 days give an extensive or a complete reaction within 24 hours. This medium without peptone gives good results, but a few species, especially *C. tetani*, grow sparingly.

This is essentially the medium recently described for toxin production. As will be noted later, the same culture may be used to measure gelatin liquefaction and toxin production.

(*d*) *Production of hydrogen sulfide:* Most if not all species of gas gangrene organisms produce at least a trace of hydrogen sulfide if a suitable substrate is supplied and a sufficiently sensitive indicator is used. Media containing appreciable amounts of sodium thioglycolate, cystine, glutathione and probably other sulfhydril-containing compounds yield considerable hydrogen sulfide with all species tested. On the other hand, mediums containing 1% to 2% of peptone rich in organic sulfur, such as proteose peptone, and one of the usual forms of iron as an indicator show darkening with all species tested. Spray (1936)[30b] apparently recognized these facts and developed a rather curious lead acetate medium, which we have been unable to reproduce with any uniformity.

Under these circumstances it seemed desirable to select a medium which will give a clear-cut positive reaction with a large to moderate yield of hydrogen sulfide

and would give a negative reaction with minute traces. Two media fulfill these specifications fairly satisfactorily, producing readable reactions in 24 hours or less which agree moderately well with the conflicting records concerning the several species.

A.

Proteose peptone	20 gm.
Sodium phosphate (Na_2HPO_4)	2 gm.
Dextrose	1 gm.
Agar	2 gm.
Water	1000 cc.

Dissolve, adjust to pH 7.6 and add 10 cc. of 2% lead acetate. This results in a cloudy precipitate which, however, remains after autoclaving in reasonably stable suspension.

B. Modified from Hunter and Crecelius (1938).

Proteose peptone	20 gm.
Sodium phosphate (Na_2HPO_4)	2 gm.
Agar	1 gm.
Dextrose	1 gm.
Water	1000 cc.

Dissolve, adjust to pH 7.6 and add 10 cc. of 1.5% bismuth and ammonium citrate. This ordinarily produces a solution which remains clear after autoclaving.

(*e*) *Formation of indol:* The sugar-free base (described under media for sugar fermentation) serves satisfactorily as a test medium for indol formation, but the following mixture gives more consistent results:

Bactotryptone	20 gm.
Sodium phosphate (Na_2HPO_4)	2 gm.
Dextrose	1 gm.
Agar	1 gm.
Sodium thioglycolate	1 gm.
Water	1000 cc.

All indol formers in the group give in this medium strongly positive reactions in 24 hours with Ehrlich's reagent.

It has been shown by Reed (1942)[78] that certain species which give negative reactions for indol do so because the indol is broken down as rapidly as formed. This is not considered in the tabular statements (Table 1). All species which give a negative reaction for indol are included among those which do not form indol.

(*f*) *Reduction of nitrate:* Cultures at 24 hours in the following medium give clear-cut reactions for nitrite with Tittsler's (1930)[93] sulfanilic acid, dimethyl-α-naphthylamine reagent:

Bactotryptone	20 gm.
Sodium phosphate (Na_2HPO_4)	2 gm.
Dextrose	1 gm.
Agar	1 gm.
Potassium nitrate	1 gm.
Water	1000 cc.

It has been shown by Reed (1942)[78] that certain species of *Clostridium* fail to reduce nitrates; others reduce nitrates to nitrites, and still others reduce nitrites as formed to ammonia. In this paper, however, the former and the latter are grouped together, as has been done in the past, as species which fail to accumulate nitrites.

(*g*) *Decomposition of protein:* The active proteolytic species give readable reactions in 24 to 48 hours on a variety of media. Less active species give indefinite results for long periods. The most rapid results have been obtained with a medium consisting of equal parts of one of the plating media (described above) and skim milk, in plates. The milk is prepared as described in (*a*).

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PREVENTIVE MEDICINE AND EPIDEMIOLOGY

UNDER THE CHARGE OF
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THE FUNCTIONS OF HYPOTHESES IN EPIDEMIOLOGY

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MANY definitions of Epidemiology found in medical literature or heard in discussions of the subject tend to restrict this field of medical science to the use of particular methods, or to reflect some particular kind of disease problem under study. Others, such as the statement that epidemiology is the natural history of the disease, are so general that they fail to define just what is involved.

Recently, the editorial question in the "*American Journal of Public Health*,"[1] "What and who is an epidemiologist?" called forth a running stream of definitions. Most of these were busied with demands for partic-

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ular personalities, qualifications, skills or techniques, for establishing facts in one situation or another, or in one disease or another. All in all, they had the effect of limiting epidemiology to the doings of a few rare individuals and using relatively few special skills, rather than leaving elbow room for the pursuit of the real objective in whatever manner, or by whoever may be suited for the purpose.

Rather than Who is an epidemiologist? we should ask What is epidemiology?* One says it is the investigation of the extent, source and control of an *epidemic*, another, that it is the study of *past* epidemics. Some define it as the *statistical* study of disease. Still others set it up not as a science, but as a sort of mystery to be unraveled, either by the gumshoe or shoe leather method.[1] But John Bard in 1749, before the Weekly Society of Gentlemen of New York said, "Of all the subjects on physical knowledge there are none so amazing or which afford more useful speculation than the history of epidemical diseases. By epidemical diseases are meant general or spreading disorders attacking great numbers within the circle of its appearance at or near the same time;—proceeding from the contagious affection of the air and sometimes propagated by a contagion received from one, to another person unaffected."[2] Nearly 200 years later Frost said, "Epidemiology is something more than the total of its established facts. It includes their orderly arrangement into chains of inference which extend more or less beyond the bounds of direct observation." This modern version of Bard's "useful speculation" is probably most important of all, for the very magnitude of the phenomenon under study is reason for extending beyond the bounds of direct observation. Epidemiology is an attempt to understand disease, not so much as it affects the individual or as it behaves at a given time or place under the eye of the observer, but rather as it imposes itself upon whole groups of people without limits of size, time or space. The circumstances falling between cases may be of more significance than the cases themselves. In clinical medicine, the case begins with the onset of sickness, and terminates when the patient is feeling well again; but in epidemiology, the case begins when the patient is infected, and lasts as long as he is infective. In certain diseases the two may closely coincide, but in many, they may be widely or even totally divergent, for in certain diseases, even the majority of infected and infective individuals never have any sickness at all. In others, infectiousness may precede or long outlast the clinical disease. Thus, for epidemiologic purposes, the silent periods—the incubation period, infectious period or healthy carriage—may be even more important than the manifest illness. Again, many of the features of disease movement in a population are not amenable to direct observation, and even if they are, it is only on small samples from which inferences must be drawn in regard to the whole.

Disease can be seen when and where it occurs, but its infectious agent cannot be seen when it is spreading. For this reason, epidemiology to a considerable extent has been the formation of inferences in regard to how infectious agents spread from the distribution of disease which results from it. Time and place relationships between cases are the "established facts" which afford the basis for "chains of inference." Hirsch's *Historic and Geographic Pathology* in its time was epidemiology at its finest, for

* One is tempted to answer that epidemiology is "the study of epidemic disease;" thus including all pertinent phases, descriptions, emphases, methods, subdivisions, philosophies, hypotheses, facts, modifying circumstances, and narrower concepts that improperly limit and confuse the answer.—EDITOR.

without the aid of the as yet unborn science of microbiology he painstakingly recorded the distribution of disease and drew inferences from it as to *how* its microbic agent was spread. From the point of view of present day knowledge, his inferences were often discrepant, but usually only insofar as knowledge of the fields into which they lead were discrepant. For example, his conclusion that malaria was due to "processes of decomposition set up in organic matters" was right, broadly speaking, as far as it could go in 1880. He correctly associated the disease with something developing in water under particular conditions. Knowledge of just what was brewing in the water had to wait advances in knowledge in the field of "decomposition of organic matter" itself. But the conditions defined by him under which malaria occurred might be recognized at once in the light of present knowledge as those under which *Anopheles* thrives.

However orderly such chains of inference may be, their correctness is dependent in part on the weight of the circumstantial evidence on which they are based, and in part on knowledge in the fields into which their interpretations lead. As Frost well stated it, "The theories of epidemiology which have been recorded at successive times have been limited in their approach to present conceptions, not merely by the limits of the facts known at the time, but equally or even more by the status of contemporary knowledge and thought in other fields, since this has largely governed the interpretations given to the facts."

The function of a hypothesis in epidemiology is therefore twofold: (1) to arrive as near a conclusion as possible through preponderance of circumstantial evidence; but, (2) perhaps more largely and equally important, to form a plan or pattern to give direction to subsequent studies, or to carry a question which has been raised in the course of studies in one field of science across the boundary to other fields for further investigation.

The ideal manner of progression in furthering knowledge would be to form a hypothesis from initial studies to be borne out, amended, modified or rejected in accordance with each additional investigation in the fields into which it has led; and only when the sum total of the evidence has become conclusive, to use the legal expression, beyond a reasonable doubt, or the hypothesis has been thoroughly tested or suitably amended by tests in other fields to allow it to be accepted as a conclusion or doctrine. An example of this sort of sound hypothesis is seen in Hirsch's compilation of data concerning the history and geography of malaria, culminating in the statement that, "We shall not proceed far in explaining the endemicity of the fever without the assumption of a material and specific poison, the development of which depends on processes of decomposition of organic matter under the conditions laid down in the 'swamp theory'."

How well Hirsch succeeded in establishing facts can best be shown by the following quotations from his discussions. "Among the factors which determine the occurrence and diffusion of the malarial diseases, climatic and telluric conditions hold first place. The dependence of malaria production on climatic influences, of which the geographic distribution over the globe has already given us indications, is brought out in the most definite way by the prevalence of malarial fever (1) at certain seasons, and (2) under certain meteorologic conditions, particularly under the influence of heat and atmospheric moisture. The question here raised leads us to inquire into the influence on the production of malarial disease exerted by those factors that are characteristic of the climate by conditions of heat

and moisture. That inquiry rests directly on our knowledge of the geographic distribution of malaria, and the facts given in the course of the foregoing sketch find a definite expression in the law that the disease shows a progressive decrease both in extent and intensity from the equator to the poles, and that there is a certain limit beyond which it does not occur either endemically or epidemically, or if it does occur, then only in cases imported.

"A rather high temperature, therefore—and all observers agree in this opinion—forms an essential condition for the development of malaria. A confirmation of this is found not only in the circumstance that the extent and intensity of the disease in malarious foci at the several seasons of the year are in direct proportion to the height of the respective temperatures, but also in the fact that the great epidemics or pandemics have been immediately preceded by hot years, or have coincided with them. But although there can be no reason to question the general importance of high temperature for the production of malaria, there is just as certainly another series of facts which serve to keep that significance within due limits.

"An influence not less pronounced than that of temperature is exerted by the degree of atmospheric moisture, or of the atmospheric precipitations that result therefrom. But inasmuch as this etiologic factor really deals with the saturation of the soil caused by the precipitations and with the fact that the saturation, when complete, sets limits to the development of malaria, then it is self-evident that we are concerned here with a certain measure, or with the relative amount of precipitation. The fall must be all the more copious, if it is to aid in the production of the disease, when the soil is naturally dry; while, on the other hand, a very heavy fall on a naturally wet soil will prevent the development of the disease till such time, at least, as the soil has again become in a measure dried through the evaporation or sinking down of the moisture within it. But there are still many unexplained exceptions to the rule which serve to show how far we are from having a complete insight into this factor, and it is only through careful analysis of the facts that we shall succeed in letting some light in upon the still very obscure region of etiology.

"The endemic and epidemic occurrence is affected by altitude and configuration of the ground under the general law that the extent and severity of malarial disease diminish in proportion as we ascend above sea level or as we move from level to hilly country. On the one hand, the mean summer temperature is a determining factor; the height to which malaria ascends is in definite ratio to the geographic position, and accordingly in an inverse ratio to the latitude of the place. The second factor in this relative immunity of elevated localities is undoubtedly the state of the soil as regards moisture, which is naturally different from the degree of moisture in the plain. Wherever malaria is endemic at more or less considerable elevations, the seat of the disease is always a valley with a small declivity or a basin-like depression in a plateau.

"An important aspect is undoubtedly that which presents itself in the question how far the disease depends upon the geological and physical conditions of the soil. Any evidence that the geological character of the soil itself is concerned is questionable and we may limit the influence of the soil to its physical characteristics—the porosity, degree of saturation, the amount of organic detritus, and further on the tillage, and perhaps also upon the products resulting from cultivation. The chief seat of malaria is always found where there is a permeable and highly hygro-

scopic clay soil, and least favorable of all is a sandy soil, readily open to saturation, but unable to retain the absorbed moisture, and becoming dry almost as soon as it is saturated. Under all these circumstances, then, the malaria-producing property of soil seems to depend on saturation of the ground.

"In the second place, we find malaria exceedingly common in small and often definitely circumscribed spots by the sides of lakes, small streams or brooks, pools, ponds and ditches (and extending just as far as the basin makes its influence felt in saturating the soil of the neighborhood).

"The amount of organic matter in the ground (or ground water) is the last of those properties of the soil which we have found to stand in relation to the origin of malaria."

And how well Hirsch fulfilled the second purpose of a hypothesis is shown by his statement pointing the finger of suspicion to a set of conditions now known to be those favorable to breeding of *Anopheles* mosquitoes. As he summarized it, "The close association of malaria with a particular kind of soil, highly saturated and rich in organic matter, and the fact that the disease breaks out whenever that kind of soil is subjected to a high temperature suggest the conclusion that the development of the morbid poison goes hand in hand with the processes of decomposition set up in organic matters under those circumstances."[6]

Particularly where these hypotheses are formed well in advance of developments in the specific fields into which they lead, they are likely to become less and less hypothesis and more and more doctrine or dogma. And, having become dogma, subsequent investigations are likely to take the form of attempts to support the hypothesis in favor; and we frequently witness amendments, modifications, or rejection of evidence (or its interpretation) when new evidence is not in line with the doctrine rather than a change in the hypothesis. Against the theory of the origin of pellagra from the consumption of decomposed maize, two important objections were encountered. But instead of bringing about a modification of the maize hypothesis—already become a doctrine—the conflicting observations themselves were ingeniously interpreted so as to be brought in line with the favored hypothesis. The first difficulty was the fact that maize is largely cultivated and used as a food in many areas in which pellagra was quite unknown. The proponents of the maize theory argued that in all these areas the corn was either thoroughly dried, thereby preventing decomposition; or the damaged maize was not eaten by man but made into food for the hogs. The second objection—the occurrence of sporadic pellagra which could in no way be associated with maize—was answered either by the assumption that other kinds of grain could under certain circumstances suffer changes like those of maize, or that such cases were so different, "not only in etiology but also in the nature and concatenation of the symptoms, that the judicial standpoint of all the more recent observers was that there never was more than a resemblance between them and the endemic disease. Arbitrarily made up groups of symptoms included nervous or psychical affections, with disorders of the digestive organs and with morbid appearances of the skin; and, after full collection and thorough scrutiny of all the observations relating to the matter, all recent observers found it necessary to speak of pseudo pellagra."[7]

But when the accumulation of evidence finally forces a change in hypothesis, the tendency has seemed to be not to amend or modify an existing hypothesis, but to launch a new one even in the face of large accumulations of evidence in favor of the older one. Perhaps one reason

why it is so easy to launch a new hypothesis is that an older one, however much soundness it may contain, not having afforded a practical solution of the disease problem, becomes wearisome and is easily set aside because of the hope offered by a new one. "It is not easy when divergent theories are presented to distinguish immediately between those which are sound and those which are merely plausible." (Frost.) And this has been especially true when epidemiologic inferences have led into fields not yet well bounded or cultivated. Favor veers from one to another with each new set of observations. Any former hypothesis in time comes to have its adherents, who continue to stress supporting evidence as well as inferences from favorable findings, remaining faithful to the old and rejecting the new. Meanwhile, the newly advanced hypothesis acquires followers—the more rapidly as it appears more promising—until, having gained enough to constitute "a strong minority," we witness the all too frequent situation of two opposed schools; each bending its efforts, not so much to the study of the problem as to the support of its own group, with one or the other gaining or losing with developments in one field or the other. And it is difficult, with the growth of opposing schools, even with acquisitions of knowledge, not to be swayed by preferences for one or the other existing doctrine, or to view both the opposed hypotheses dispassionately. Finally, and frequently because of the impetus of some particular development in knowledge, the more hopeful, newer hypothesis completely overthrows the older.

It frequently happens that, where there are two opposed concepts, it will be found that they were derived at different times under the influence of contemporary developments in one science or another; not so much from conflicting observations, as from resisting inferences evolved from essentially similar observations. And it is frequently the case that these resisting inferences were formed long in advance of developments in the specific fields involved. In the light of subsequent advances, it is seen that the approval of either concept necessitates the acceptance of assumptions of doubtful worth in the theory favored, and the rejection of valid evidence in the opposition.

Therefore, it is constructive to turn back to the data from which earlier concepts have been evolved in order to re-test them in the light of present knowledge, to seek out the discrepancies in each and the validities in both, which become more discernible with development of knowledge in the fields in which they lie. Thus, erroneously drawn propositions may be amended, or the validities in conflicting hypotheses which were formed from essentially the same type of observations may be proved adjustable into a different concept.

The changes which have taken place at different times in concepts of the epidemiology of leprosy well illustrate the manner in which hypotheses rapidly become doctrines, how new observations are rationalized to fit the favored dogma, and how older hypotheses are finally thrown over by newer and more hopeful ones. And, finally, it may be seen how by viewing the two hypotheses in leprosy, rejecting the assumptions of doubtful worth in both and accepting the validities in each, we arrive at a third hypothesis with which all the observations are in more perfect accord than with either of the two conflicting hypotheses.

The two opposed theories, contagion and heredity, have prevailed at different times in leprosy. These concepts were derived not so much from conflicting observations as from resisting inferences evolved from essentially similar observations. Both were formed long in advance of develop-

ments in the specific fields of science involved, and with acquisition of knowledge in these fields, discrepancies in each and validities in both become apparent. The approval of either concept necessitates the acceptance of assumptions of doubtful worth in the theory favored and the rejection of valid evidence in the opposition.

In the light of modern knowledge, it can now be seen that the familial tendency in leprosy upon which Danielson and Beck based their theory of heredity is not that of a hereditary disease—that is, it is not Mendelian; but, none the less, there is a striking familial tendency. It can likewise be seen that the long continued selectivity seen in family lines is not compatible with any known law of contagion, the theory which gained preference through the discovery of the Hansen bacillus. There is no longer any question as to the actuality of both familial tendency and contagion in leprosy. The validities as well as the discrepancies in both theories can best be harmonized by a third concept—that leprosy occurs as a result of contagion to be sure, but in the exposed who are hereditarily susceptible to the infection.[2]

In the earlier part of the nineteenth century, the formation of hypotheses from the facts relating to disease distribution was the only tool available to epidemiology. But this method yielded masterpieces of inference such as Budd in typhoid fever or Panum in measles. But in the latter part of the century, hypotheses lost ground and experimental proof was exacted. Finlay's long held *Stegomyia fasciata* hypothesis in yellow fever[5] was not established (he attempted experimental transmission) until, aided by Carter's demonstration of the extrinsic incubation period,[4] Reed was able to set up an appropriate transmission experiment with *Aëdes ægypti*.[9, 10]

Since epidemiology is so largely an inferential science, it is natural that hypotheses based on numbers of features or on a variety of findings, in other words on a preponderance of evidence, are more likely in general to point in the right direction than those based on some single feature. For example, the fallacy of the once common idea that seasonal prevalence of disease is necessarily an indication of mode of spread is now well known. The error in the generalization that diseases transmitted through upper respiratory contact are diseases of winter because of "crowding" and insect-borne or gastro-intestinal diseases necessarily occur in the opposite seasons because of summer increase in transmission, may be seen in the fact that no two of the upper respiratory diseases have the same seasonal curve. Diphtheria begins its increase in August, while whooping cough or meningococcus meningitis may remain epidemic well into summer. On the other hand, the insect-borne typhus (for obvious reasons, of course) is a disease of winter.

Perhaps it is because laboratory findings or experiments are in a sense more easily visualized than more or less laborious collections of data on disease distribution that some single experimental feature of disease so often takes preference in the formation of epidemiologic inferences over any amount of distributional evidence to the contrary. The older inference that hookworm infestation was due to drinking water,[7] based on the finding of hookworm eggs in the fecal discharges of patients could hardly have been made if the difference in incidence in those wearing shoes and those going barefooted had been considered.

The earlier history of the development of present day epidemiologic concepts is filled with erroneous hypotheses which have prevailed at one time or another. From the point of view of present day knowledge, it may be difficult to see how some of these could have evolved, until it is

remembered that in addition to reasons already stated—chiefly inadequate knowledge of fields into which the hypotheses lead, many of them were based on observations limited to the locality which could be covered by the single observer and without the aid of information concerning the behavior of the disease elsewhere. The first outbreak of poliomyelitis recorded in its epidemiologic aspects in this country was in Rutland County, Vermont, in 1894. The recorded outbreak extended only as far as Caverly could travel in a horse and buggy. Only recently this restricted occurrence was cited as having special epidemiologic significance, in spite of the fact that it is now well known that the outbreak was by no means confined to the area covered by Caverly's study. To be sure, there are local investigations which are classics in epidemiology—Budd on typhoid, and Snow on cholera—but more often, failure to view disease broadly has led to erroneous concepts. There are diseases which occur only in restricted areas, not because of any effort to keep them there, but because it is only in these areas that they find the necessary conditions for propagating themselves. For these, the cause is to be sought in local conditions. But for most, it is clear that disease in one people cannot be prevented, nor even understood, without taking into account its behavior in other places. We may still argue with Boswell and Johnson whether epidemic disease comes into St. Kildes on the wind, or on the ships which make the harbor with the favorable wind, but we know that epidemic disease, lurking in insects, goods and chiefly in the healthy carrier, recognizes no boundaries. In the past, distance itself aided in protecting one from the diseases of another. But the isolation of the past is a thing of the past, for microbes in their way enjoy the speed of modern travel as much as we do in our way. Thus the cause of most diseases is not local but is due to a condition or conditions in many areas. Study of the circumstances under which it travels or attention to conditions which can be associated with variations in its occurrence in different localities, may be more revealing than studies, however intensive, in one area.

The facts have sometimes been assembled by the traveling investigator, such as Panum in the Faroes; and Hansen, the Norwegian discoverer of the bacillus of leprosy, who in 1888 journeyed to Minnesota in the United States to investigate the disease in American-born descendants of Norwegian lepers. More often, they have come from the automatic merging of the findings of many workers in many lands. As with the milkmaid who assured Jenner that she was immune to smallpox because she had had cowpox, they have been given to the world by the folklore of simple people.

A quarter of a century after the epidemiology of yellow fever had been thought to be finally settled by Reed's successful transmission of the disease, foreshadowed by Finlay's hypothesis in Havana and made possible by Carter's establishment of the extrinsic incubation period in Orwood, Mississippi, the next important extension in knowledge of the epidemiology of yellow fever came with the establishment of a field laboratory by the Rockefeller Foundation in Lagos, Nigeria. Isolation of the virus and a blood test revealed that the disease was not limited to small areas on the west coast of Africa and east coast of Brazil, but that the virus actually was distributed from Dakar and Angola on the west coast, to Uganda and the Ethiopian foothills. In the Amazon valley, the belt of prevalence was extended to parts of Venezuela, Colombia, Ecuador, Peru, and Bolivia as well as Brazil, and a new species of mosquito was found to transmit the jungle type of yellow fever. Methods of

control of the fever carried by this sylvan *Hæmagogus capricornus* were entirely different from those for the city dwelling *Aëdes ægypti*, the mosquito which had been supposed to be the sole distributor of the disease.[8]

Thus, the epidemiologic knowledge of disease has been assembled from hypotheses originating in studies in all parts of the globe. Formerly, great epidemics followed large migrations of people and the development of new trade routes. Two striking examples are the dissemination of plague from Hong Kong by trading ships, and the breaking loose of Asiatic cholera from what was considered its native habitat to spread over Europe, Canada, and the United States in 1830. Some governments disregarded medical regulations entirely or even refused to admit the occurrence of epidemic disease within their own borders for fear of interrupting trade with others. Individual nations or cities attempted to check the entrance of disease by drastic measures at their ports and land borders. Some port authorities burned whole ships and their cargoes which had arrived from infected countries, or detained passengers and crew for long periods of quarantine.

With nations as with individual communities, the older idea of throwing the sick into pesthouses by law and under guard has given way to coöperation. We have learned that pestilential disease does not obey our laws but follows its own, traveling from one people to another, as we give it opportunity with our own travels. In the earlier decades of the 19th century, international coöperation in matters of health was instituted by the countries bordering on the Mediterranean Sea. The Turkish government, in 1839, invited representatives of other nations to meet with the Sanitary Commission of Constantinople for better regulations for ships with disease entering that port. This Commission extended its supervision to the ports of the Black Sea, the Straits, Asia Minor, and the Red Sea, and it provided sanitary regulations governing the pilgrimage to Mecca. The Egyptian Council assumed responsibility for the pilgrims of that region and after the opening of the Suez Canal (1869), it undertook medical supervision of traffic through that waterway.

In 1851, the first of a series of International Health Conventions was called in Paris. At this, and subsequent meetings in Constantinople, Vienna, Washington, Rome, Dresden, Venice and Paris, regulations were formulated concerning the notification of diseases such as cholera and plague, the medical inspection of departing crews and passengers from ports where certain diseases were known to exist; inspection and quarantine of those arriving from infected countries; and examination of certain types of cargoes likely to carry disease.

At the meetings of 1903 and 1907, the Office Internationale d'Hygiène Publique was established in Paris, and has continued to standardize methods of control of epidemic disease as between nations. It had the authority and financial support of 51 nations, and has been of great service in the field of international health. It has largely had to do with unifying the many national laws of sanitation. It has coördinated the health laws concerning pilgrimages from three continents to Mecca to prevent the exchange of cholera. It has aided in providing medical facilities in ports of all parts of the world for the treatment of sailors—in short, the international coöperation promoted by the Office d'Hygiène Publique can, perhaps, be best visualized in the large disks that can be seen on the hawsers of ships in port in all parts of the world, put there by mutual agreement to prevent the debarkation of rats which are the carriers of the bacillus of bubonic plague. Finally, the Health Section of the League of Nations was formed

to convene international conferences to exchange information and to take steps necessary to insure an effective international coöperation in matters of health. The Health Section of the League is the culmination of the efforts toward concerted action in preventing disease. It has made its influence felt in all parts of the world in combating epidemics, in standardizing medical biologic products, and in the prompt dissemination of information concerning the prevalence of epidemic disease everywhere.

Just as epidemiologic knowledge has been assembled from all parts of the world, it has been redistributed in its application to the prevention of disease by international coöperation at its best. International coöperation has not failed in this because the objective, freedom from disease, has been one on which all have agreed. No one anywhere gets sick by preference. The signal flag flown for quarantine inspection of ships is the same everywhere.

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BOOK REVIEWS AND NOTICES

A Handbook of Medical Library Practice. Editor, Janet Doe. Compiled by a Committee of the Medical Library Association. Based on a Preliminary Manuscript by M. Irene Jones. Pp. 609. Chicago: American Library Association, 1943. Price, $5.00.

Medical libraries have a number of special needs that will be met by this long-needed and long-contemplated handbook. Especially in the case of the smaller libraries, the librarian who starts better equipped with library training than with medical knowledge will benefit from the book as much as will his or her confrère who has been trained contrariwise. Of the 8 chapters, most are concerned with such matters as selecting and ordering, cataloging, handling of pictures and microfilms. There is a valuable final chapter on reference work, with an extensive Annotated List of Reference Books; the subject is so vast, however, that even its 174 pages cannot adequately cover the wide range. For instance, of 2 subjects taken at random, "Bibliographies of Aviation Medicine" includes but 2 items, neither of them being Fulton's. "Hæmatology (Encyclopedias, etc.)" includes 3 items: Downey, Kracke, Hirschfeld (in this order), Kracke however being but one of many 1-volume textbooks on the subject.

Especially interesting to the Reviewer is the chapter on Rare Books and the History of Medicine. More than half of the 115 pages is devoted to an Annotated Bibliography of books useful in a historical medical collection. As is fitting for a librarian's production, the book has a full and apparently accurate index.

Miss Doe and her 10 colleagues are to be congratulated on the production of a volume that is a valuable reference source to those interested in medical literature, and history, as well as an invaluable desk book for medical librarians.

E. K.

Medical Genetics. By Laurence H. Snyder, Sc.D., Professor of Medical Genetics, Ohio State University. Pp. 130; 24 figs. Durham, N. C.: Duke University Press, 1941. Price, $1.50.

The text material in this small volume is taken from a series of lectures presented to the medical schools of Duke University, Wake Forest College, and the University of North Carolina. Its contents include the study of human heredity, medico-legal applications, cancer, hereditary disorders of the different bodily systems, and a final chapter on the future development of medical genetics. It should aid in both the diagnosis and prevention of inherited diseases and abnormalities, and in giving correct advice to prospective marriages and pregnancies. All in all, it is a very satisfactory and useful compilation of data on this subject, and certainly a volume to be highly recommended to all clinicians and medical students. The emphasis rests on inherited abnormal states, whereas the principles of genetics are discussed only to the extent of giving us a concise understanding of the mechanisms involved.

M. T.

Atlas of Obstetric Technic. By Paul Titus, M.D., Obstetrician and Gynecologist to the St. Margaret Memorial Hospital, Pittsburgh; Secretary, American Board of Obstetricians and Gynecologists. Illustrations by E. M. Schackelford, Medical Illustrator, John C. Oliver Memorial Research Foundation, St. Margaret Memorial Hospital. Pp. 180; 193 figs. St. Louis: C. V. Mosby Company, 1943. Price, $7.00.

It is said that a picture is worth a thousand words. On this assumption Titus's atlas with its 193 carefully selected illustrations should be a considerable

addition to textbooks dealing with obstetrics. In this volume the author has covered a wide range of subjects. In addition to illustrating strictly obstetrical topics, he considers in pictorial form such subjects as major and minor operations upon the abdomen which not infrequently complicate pregnancy, as well as technical procedures required in the treatment of patients suffering from sterility. A study of the pictures in this volume should give the medical student a clear comprehension of the subjects covered. The illustrations are line drawings of quite satisfactory size, and are described by carefully considered legends. One or more blank pages for notes or additional drawings are available at the end of each chapter. D. M.

PHYSIOLOGY IN AVIATION. By CHALMERS L. GEMMILL, B.S., M.D., COM., M. C., U. S. N. R., Associate Professor of Physiology, The Johns Hopkins University, School of Medicine, Baltimore, Md.; Instructor in Physiology, School of Medicine, Naval Air Station, Pensacola, Fla. With a chapter on Instrument Flight by LT. FREDERICK B. LEE, U. S. N. R. Pp. 132; 18 figs.; 18 tables. Springfield, Ill.: Charles C Thomas, 1943. Price, $2.00.

DR. GEMMILL, now on active service, has published in an entertaining and instructive book the lectures on the physiology of aviation given in the School of Aviation Medicine, Naval Air Station, Pensacola, Fla. In brief and succinct manner he has covered the essentials of physiology related to aviation problems. An interesting historical introduction is followed by chapters on the mechanics of respiration, on gas transport by the blood, on the circulation, on temperature control, and on the physiology of muscular exercise. The principles discussed are then applied to the problems arising in flight at high altitudes. Chapters are devoted to anoxia and aëroembolism, and also one on the effects of acceleration on man. A final instructive chapter on instrument flight by Lieut. Frederick B. Lee is appended.

Anyone interested in a clear, short account of the medical problems in this interesting field should read this book. W. S.

ESSENTIALS OF GYNECOLOGY. By WILLARD R. COOKE, M.D., F.A.C.S., Professor and Head of the Department of Obstetrics and Gynecology, University of Texas. Pp. 474; 197 illus., including 10 in color. Philadelphia: J. B. Lippincott Company, 1943. Price, $6.50.

THIS volume is a condensed textbook prepared for beginners in the subject. The various topics, though necessarily treated briefly, are satisfactory for the purpose. The illustrations include a number of excellent line drawings in addition to photographs of gross and microscopic specimens.

The material is well arranged and covers the clinical as well as the more academic aspect of the various subjects. The book is completely without reference to other works on the same subject. It should be of service to the medical student in securing the essentials of gynecology. D. M.

CONSTITUTION AND DISEASE. By JULIUS BAUER, M.D., Professor of Clinical Medicine, College of Medical Evangelists, Los Angeles, Calif.; formerly Professor of Medicine, University of Vienna. Pp. 220; 5 illus. New York: Grune & Stratton, Inc., 1942. Price, $3.50.

THROUGH this small volume, Dr. Bauer has made his Viennese lectures on "Endocrinology and Constitutional Pathology" available to the American medical professions. After discussing the rôle of constitutional factors in the etiology of various disease processes in general, he considers the various constitutional biologic inferiorities of the organs and tissues of the body that may be expected. The most important chapter is that which deals with diseases

having chiefly a constitutional factor in their etiology, such as diabetes mellitus and diseases of the hemolytopoietic system.

Dr. Bauer finally lays down certain principles of treatment based on the defective constitutional etiology and points out certain pitfalls which one may encounter when these deficiencies are not adequately considered.

The real virtue of the book lies in its ability to stimulate thought as to the constitutional deficiencies which exist as part of the ordinary medical problems which confront every practitioner and as to how he may use these factors to modify his treatment to the patient's advantage. This book is highly recommended both to those actually engaged in the practice of medicine and to medical students. J. S.

MODERN TRENDS IN OPHTHALMOLOGY. Edited by FREDERICK RIDLEY and ARNOLD SORSBY. Pp. 699; 217 figs; 8 color plates. First published in Great Britain, 1940. New York: Paul B. Hoeber, Inc., Medical Book Dept. of Harper & Bros., 1943. Price, $10.00.

THIS book brings various phases of Ophthalmology up to the last minute. The book is divided into 7 parts. The first part deals with the relation of ophthalmology to general medicine. In its first section on infection, allergy and immunity, of chief interest is the chapter on ophthalmologic conditions in tropical and subtropical regions. This will be of distinct value to ophthalmologists in the Armed Forces. Section 2 concerns the ocular manifestations of some general disturbances; it contains an excellent review on arteriosclerosis by Friedenwald. Section 3 deals with the ocular manifestations of some dystrophies.

The second part deals with diagnostic procedures; the third part with refraction and binocular vision; the fourth part, on the physiology of vision, contains a very good review by Selig Hecht. Part 5 groups together a number of unrelated subjects under the heading "new conceptions in pathology." Part 6 concerns modern treatment, and Part 7 the social aspects of ophthalmology. In this last chapter there is an article on chemical warfare in relation to ophthalmology.

As in compilations, some chapters are better than others, but it is an extremely valuable bibliography of the modern literature. F. A.

MANUAL OF STANDARD PRACTICE OF PLASTIC AND MAXILLOFACIAL SURGERY. Military Surgical Manual I. Prepared and Edited by the Subcommittee on Plastic and Maxillofacial Surgery of the Committee on Surgery of the Division of Medical Sciences of the National Research Council, and Representatives of the Medical Department, U. S. Army. ROBERT H. IVY, Chairman. Pp. 432; 259 figs. Philadelphia: W. B. Saunders Company, 1942. Price, $5.00.

THIS is the first of a series of Military Surgical Manuals prepared under the auspices of the National Research Council for use by the Army and Navy as guides in the management of war casualties. The subject Plastic and Maxillofacial Surgery is very ably discussed. The text is divided into 4 parts: reconstructive surgery, maxillary surgery, maxillofacial prosthesis, and anesthetic technique. The subjects, although necessarily brief, are clearly and concisely presented. The book will be read and appreciated not only by those in the armed forces, but by many in civilian practice, for it is more than a simple war-time manual. H. Z.

MEDICAL CLINICS OF NORTH AMERICA (St. Louis Number, Vol. **26**, No. 2, March, 1942). Pp. 635; 26 ills. Philadelphia: W. B. Saunders Company, 1942. Price, $16.00 a year.

THIS volume maintains the usual high level of excellence of this bi-monthly publication. In this number, there are 14 fine articles on Medical Emergen-

cies, and a symposium of 6 articles on Tuberculosis. The 212 pages on Medical Emergencies form a handbook which every physician might well wish to have at hand in time of need. It is unfortunate that the 85 pages on Tuberculosis are inappropriately included in the same volume.

It is unfair to pick on minor points for criticism, but the lines on page 460 reading "Botulism—decomposed proteins and fats often found in cream or custards affect anterior horn cells of the spinal cord" seem so misleading and inaccurate as to deserve special mention. O. P.

ABDOMINAL AND GENITO-URINARY INJURIES. Military Surgical Manual III. Prepared under the auspices of the Committee on Surgery of the Division of Medical Sciences of the National Research Council. Pp. 243; 28 figs. Philadelphia: W. B. Saunders Company, 1942. Price, $3.00.

THIS, the third manual of a series of Military Surgical Manuals prepared under the auspices of the National Research Council, is divided into two parts. The first deals with injuries of the abdomen; the second, with injuries of the genito-urinary tract. Diagnosis, surgical treatment, and subsequent care of the wounds are outlined. The text will serve to familiarize members of the medical forces of the Army and Navy with the type of wounds encountered in warfare. H. Z.

DISEASES OF THE NOSE, THROAT AND EAR. By WILLIAM L. BALLENGER, M.D., F.A.C.S., Late Professor and Head of the Department of Otology, Rhinology and Laryngology, School of Medicine, University of Illinois; and HOWARD C. BALLENGER, M.D., F.A.C.S., Associate Professor of Otolaryngology, Northwestern University School of Medicine, Chicago; Surgeon, Department of Otolaryngology, Evanston Hospital. Eighth ed. Pp. 975; 604 engravings, 27 plates (25 in color). Philadelphia: Lea & Febiger, 1943. Price, $12.00.

THIS has long been considered one of the leading text and reference books in Otolaryngology. The 1943 edition includes many new illustrations, the descriptions of some new operative procedures, the applications of the sulfonamides to Otolaryngology, as well as that wealth of information found in previous editions. It is not intended in any way to intimate by the brevity of this Review that the importance and excellence of the production is not thoroughly appreciated. K. H.

ANNUAL REVIEW OF BIOCHEMISTRY. VOL. XII. Edited by JAMES MURRAY LUCK, Stanford University; Associate Editor, JAMES H. C. SMITH, Carnegie Institution of Washington, Division of Plant Biology, Stanford University, California. Pp. 704; many tables and figs. Stanford University P. O., California: Annual Reviews, Inc., 1943. Price, $5.00.

THIS volume, in addition to reviewing the subjects covered at frequent intervals (if not annually in this series), has several chapters devoted to subjects either appearing for the first time or which have not been reviewed for several years. Among these may be mentioned a chapter on Synthetic Drugs by T. C. Daniels, another on Carbon Dioxide Assimilation in Heterotrophic Organisms by H. A. Krebs and one on The Electron Microscope in Biology by L. Marton. Particularly in the last-mentioned chapter the author treats the subject not as an annual review but summarizes the work done since the building of the first compound instruments in 1932.

It appears that the war has not seriously decreased the quantity nor quality of the work which is being done in the field of biochemistry. This volume in every way meets the high standards set by the previous volumes. J. J.

SKIN GRAFTING OF BURNS. By JAMES B. BROWN, M.D., LT. COL., M. C., U. S. A.; Senior Consultant in Plastic and Maxillofacial Injuries and Burns, E.T.O., U. S. A., Associate Professor of Surgery, Washington University, St. Louis; and FRANK MCDOWELL, M.D., Assistant in Clinical Surgery, Washington University. Pp. 204; 131 illus. Philadelphia, London, and Montreal: J. B. Lippincott Company, 1943. Price, $5.00.

DURING a time of war the subject of burns becomes exceedingly important. The incidence rises enormously, not only on account of fire and explosions in battle situations but also because of the hazards to civilian workers in military industries. For this reason one welcomes the appearance of a monograph compiling the treatment experience of two of this country's most skilful plastic surgeons and epitomizing modern concepts of the skin grafting of burns. The book contains the following chapters: Early General Care, Early Local Care, Preparation of Wounds for Skin Grafting, Application of Thick Split Grafts, Application of Free Full Thickness Grafts, Pedicle Flaps, Regional Repairs of Trunk and Extremities, Regional Repairs of Head and Neck, Homografts, Faults of Skin Grafts, Treatment of Burns in World War II. The excellent paper, large type and clear illustrations make for easy understanding of the recommended techniques. Many of the illustrations and case reports will be recognized by those who have been following the authors' writings in the surgical journals. Dr. Brown's note from the European theatre of operations in the Preface brings the timeliness of the book up to May 6, 1943, when this addendum was written.

The deficiencies of the book are minor and do not in any sense detract from the value of the material contained within it. No mention is made, for instance, of Roentgen ray burns. A description of the differences in lesions resulting from different burning agents such as heat, steam, electricity, Roentgen rays and friction would be helpful. There is but a brief discussion of the use of the dermatome, which in many hands has proved efficient, and the subject of full thickness grafts does not receive much emphasis. The chapter on faults and failures of skin grafts is far too short. No reference is made to constitutional reactions following the dusting of sulfonamides on granulating areas.

Merely as a treatise on the thick split graft, at which procedure the authors are unsurpassed, this book is worth far more than its purchase price.

I. W.

YOUR ARTHRITIS: What You Can Do About It. By ALFRED E. PHELPS, M.D. Introduction by R. GARFIELD SNYDER, M.D. Illustrations by JAMES MACDONALD. Pp. 192; 12 illus. New York: William Morrow & Co., 1943. Price, $2.00.

THIS book, a summary of present knowledge of arthritis, written for the laity, fills a definite need. It should also be known to all who treat these patients, for its recommendation will save the busy practitioner precious hours by anticipating many questions. Causes, course of disease and treatment are conservatively and thoroughly discussed.

W. S.

SYNOPSIS OF BLOOD DISEASES. By A. PINEY, M.D., M.R.C.P., Director, Pathological Department, Royal Cancer Hospital, London; Physician, St. Mary's Hospital for Women and Children, London. Pp. 120; 4 plates and several tables. Philadelphia: The Blakiston Company, 1942. Price, $2.75.

THIS is a rigidly skeletonized compendium of hematology, presented in telegraphic style. Most of the subject matter of hematology is adequately covered, if due allowance is made for the limitations of a work of this nature. Particular value attaches to the diagnostic outlines. Certain faults are apparent. The rapid style is sometimes careless and incoherent. The brief

statements are sometimes overly dogmatic. Hemoglobin is measured in percentage, but no standard value is given. It is wrongly stated that the anemia of sickle cell anemia can usually be relieved with iron and liver extract. British pharmaceutical proprietaries, unfamiliar in this country, are unfortunately frequently recommended in delineations of therapy. No mention is made of "unit" assay and dosage of liver extract in the treatment of pernicious anemia. The importance of sulfonamides, and of Rh factor, goes untouched in the discussion of hemolytic syndromes. The illustrations of the various blood and marrow cells (in color) are schematic. On the whole, however, the work is a useful "refresher," if judiciously read. A. C.

METHODS OF TREATMENT. By LOGAN CLENDENING, M.D., Clinical Professor of Medicine, Medical Department of the University of Kansas; Attending Physician, University of Kansas Hospitals; and EDWARD H. HASHINGER, A.B., M.D., Clinical Professor of Medicine, Medical Department of the University of Kansas; Attending Physician, University of Kansas Hospitals; Attending Physician, St. Luke's Hospital, Kansas City, Mo. With chapters on Special Subjects by various authors. Eighth ed. Pp. 1033; 138 figs. St. Louis: C. V. Mosby Company, 1943. Price, $10.00.

"THIS book is planned to furnish an outline of all the methods of treatment in internal medicine." What the authors have done, in effect, is to set under one cover the sections usually devoted to treatment in the standard texts on Medicine, Surgery, and some of the specialties.

Part I describes the procedures under headings such as Rest, Drugs (an epitomized pharmacology text), Anesthesia, Immunology, Allergy, Dietetics, Infant Feeding, Physical Therapy, Radiotherapy, Spas, Psychotherapy, and Miscellaneous Procedures such as Blood Transfusion, Spinal Puncture, etc. Part II discusses the application of these procedures for the specific condition.

It is difficult to know what group might find the book useful—it is too elementary for the specialist, too epitomized and "compended" for the student. The general practitioner may find it helpful as a handy reference in the management of some conditions, or in a procedure perhaps better executed by someone trained in this field. In no sense are the directions complete. Example: For abdominal paracentesis: "The patient should empty his bladder." No mention is made of catheterization. The experienced will not need the few simple directions that are included, while the neophyte will not think of the important step of being sure the bladder is empty by catheterization. No mention is made of recording blood pressure for impending circulatory collapse during the procedure, the use of the abdominal binder, or the procedure to be carried out should the trocar enter bladder or bowel. The use of the oscillating bed is advised, without critical comment, in the treatment of congestive heart failure. Venesection is dismissed, after a short historical account, with the statement "It may be done in pneumonia, uremia, hypertension, and in the premonitory signs of cerebral apoplexy." Indications, amount, contraindications, results, etc., are omitted. The technique of instilling iodized oil into the trachea is described for use in the Roentgen diagnosis of bronchiectasis. The action of radium on cancer is discussed in generalities, as is the entire chapter on Radiotherapy, admittedly for student and general practitioner, not for the radiologist. The author's style recalls the senior author's entertaining "Human Body," a popular style full of anecdotes, but not of great value for the physician and lacking notably in essential detail.

There is little in the book that could not be read to better advantage in current standard works. However, in the words of the authors—"All therapeutic procedure is brought together within the compass of one volume. Nowhere else, so far as we know, is such a plan carried out. It is the only justification we have for the book's existence." The text is therefore recommended, but with reservations. E. W.

WHOOPING COUGH. By JOSEPH H. LAPIN, B.CHEM., M.D., Adjunct Pediatrician, Bronx Hospital; Associate in Contagion, Riverside Hospital for Contagious Diseases, New York. Pp. 237; 25 tables, 40 illus. Springfield, Ill.: Charles C Thomas, 1943. Price, $4.50.

MUCH interest is being shown currently in whooping cough, despite, or perhaps in part because of, the refractoriness of this affection to the sulfonamide drugs. Here is a new monograph of medium size, readable and well balanced, condensing what is known and emphasizing what remains unknown. The author is a pediatrician with a wide personal experience with the disease, both in the clinic and the laboratory. He presents stock-taking chapters on history, epidemiology, bacteriology, pathology, immunology, serology, clinical manifestations, hematology, roentgenology, complications, diagnosis, prophylaxis of non-exposed children, prophylaxis of "contacts," specific treatment, nonspecific treatment, treatment of complications, and public health considerations. Every chapter is divided into well-organized subsections, and all material is presented from the practical clinical point of view.

With regard to the controversial value of vaccine prophylaxis for building active immunity, the author summarizes the results of 26 studies by different workers. According to the best evidence, immunization will reduce the attack rate to from 10% to 35% of that for unvaccinated controls. The communicability rate for intimate sibling exposures is about a third of the figure for unvaccinated controls. There is general agreement that attacks which occur in vaccinated children are markedly lighter than in unvaccinated children. The duration of immunity is still in doubt, but is probably several years, at least. Vaccines are apparently best prepared from *H. pertussis* in Phase I. These are freshly isolated strains with smooth colonies, virulent for animals, and containing in their surfaces an antibody-stimulating antigen which becomes completely lost upon repeated subculture. Of the specific forms of treatment, human sera, convalescent or hyperimmune, have received more encouraging reports than have animal sera or vaccines prepared from the whole Bordet-Gengou bacillus or its fractions.

Bacteriologic examination with the well-known cough plate method is still the most valuable single laboratory aid in the catarrhal stage of the infection. The difficulty, however, in getting a vigorous cough in infants at the proper times has led to poor success in diagnosis with this age group. Bradford and others have introduced recently the taking of nasopharyngeal swabs in suspected cases during infancy, and their results have been highly favorable.

Well-chosen illustrative data, excellent photographs and carefully selected bibliographic references contribute to the value of this review. I. W.

NOXIOUS GASES and the Principles of Respiration Influencing Their Action. By YANDELL HENDERSON and HOWARD W. HAGGARD, from the Laboratory of Applied Physiology, Yale University. American Chemical Society Monograph Series. Second ed. Pp. 294, various tables. New York: Reinhold Publishing Corp., 1943. Price, $3.50.

THIS revised edition is an excellent treatise covering the many aspects required in a thorough consideration of the noxious gases. These gases are classified according to their chemical composition and by their physiologic actions. Their list is extremely comprehensive and includes anesthetics and volatile drugs, as well as many other substances seldom embraced in the category of noxious gas.

Among the subjects treated are the laws of gases and vapors, complete with tables and formulæ for the calculations of specific problems involving them. Finally, the clinical manifestations resulting from the inhalation of each gas and the therapeutic methods for combating them are discussed.

This work is remarkably inclusive and its material is excellently presented. It is a valuable reference volume for physicians in general and should prove indispensable to those harried by industrial problems. C. C.

METHODS FOR DIAGNOSTIC BACTERIOLOGY. By ISABELLE G. SCHAUB, A.B., Instructor in Bacteriology, Department of Pathology and Bacteriology, The Johns Hopkins University School of Medicine; Bacteriologist in Charge of the Diagnostic Bacteriological Laboratory of the Woman's Clinic, The Johns Hopkins Hospital; Instructor in Bacteriology in the Nurses Training School; and M. KATHLEEN FOLEY, A.B., Bacteriologist in Charge of the Diagnostic Bacteriological Laboratory of the Medical Clinic, The Johns Hopkins Hospital. Second ed. Pp. 430. St. Louis: C. V. Mosby Company, 1943. Price, $3.50.

THIS is a new edition of the manual of bacteriologic procedures and techniques as practised at the authors' hospital. In the 200 pages of text, the procedures for the isolation and identification of human pathogens are well outlined. The book contains 3 parts: I. Bacteriological Diagnosis, II. Serological Diagnosis, and III. Media, Stains, Reagents and Tests. A new chapter (Chapt. 2) has been added—the rapid identification of pathogens by colony morphology.

The authors stick mainly to one procedure for each test—a tried and true one. Many laboratories might find this manual inadequate for their needs. Especially true would this be in the Wassermann reaction, other complement fixation tests and flocculation tests being entirely neglected.

While not covering the entire field of diagnostic bacteriology and immunology, it does bring together many proven techniques and procedures of great value to medical students, internes and technicians in diagnostic laboratories.

F. E.

ESSENTIALS OF SYPHILOLOGY. By RUDOLPH H. KAMPMEIER, A.B., M.D., Associate Professor of Medicine, Vanderbilt University School of Medicine; in Charge of the Syphilis Clinic and Visiting Physician to Vanderbilt University Hospital. With chapters by ALVIN E. KELLER, M.D., and J. CYRIL PETERSON, M.D. Pp. 518; 87 illus. Philadelphia, London, Montreal: J. B. Lippincott Company, 1943. Price, $5.00.

THIS well-written book is another in the series of "Essentials" being published by the Lippincott Company. It represents an attempt by the author to meet the needs of the practitioner of medicine in the field of syphilology.

Although the author makes no claim to originality, the material is interspersed with the results of his investigations and those of his colleagues, as well as case material from his Vanderbilt University Syphilis Clinic. On the whole, whoever, the viewpoints expressed are more or less standard.

The volume does not seem to the Reviewer to fulfill entirely the need for a text "written by a general practitioner for the general practitioner," as it does not supply, for instance, sufficient working technical details, for the relatively uninformed, of such procedures as drug mixing, technique of drug administration, lumbar puncture, and so forth.

Late syphilis, the practitioner's chief problem, is only briefly described. Little attempt is made to suggest the need for competent consultation, so that the practitioner may feel that these brief descriptions of visceral syphilis are adequate to guide him in practice.

In spite of such items, however, we regard the work as a good "refresher" text for rapid, up-to-date review of the "Essentials" of syphilology in all its phases, medical and social.

H. B.

VITAMINS AND HORMONES. Edited by ROBERT S. HARRIS, Associate Professor of Nutritional Biochemistry, Massachusetts Institute of Technology, and KENNETH V. THIMANN, Associate Professor of Plant Physiology, Harvard University. Vol. I. Foreword by E. V. MCCOLLUM, Johns Hopkins University. Pp. 452; many tables and figs. New York: Academic Press, Inc., 1943. Price, $6.50.

ACCORDING to the Editors this is the first of what is hoped to be a "succession of yearly volumes on Vitamins and Hormones which will chronicle progress

and point the way to new achievements." The subjects covered in the first volume, with the authors of each, are as follows: (1) Choline—Chemistry and Significance as a Dietary Factor, by C. H. Best and C. C. Lucas; (2) The Appraisal of Nutritional States, by Norman Jolliffe and Rita M. Most; (3) Physical Methods for the Identification and Assay of Vitamins and Hormones, by John R. Loofbourow; (4) The Chemical and Physiological Relationship Between Vitamins and Amino Acids, by H. H. Mitchell; (5) The Photoreceptor Function of the Carotenoids and Vitamin A, by George Wald; (6) The Significance of the Vitamin Content of Tissues, by Roger J. Williams; (7) Growth-Factors for Protozoa, by Richard P. Hall; (8) Physiology of Anti-Pernicious Anemia Material, by George R. Minot and Maurice B. Strauss; (9) The Intermediate Metabolism of the Sex Hormones, by Gregory Pincus and William H. Pearlman; (10) The Hormones of the Adrenal Cortex, by T. Reichstein and C. W. Shoppee.

To any one familiar with the field of Nutrition or Physiology it need not be stated that all of the authors are recognized leaders in their field. This fact is very obvious as one reads the various articles, for they are both a condensation of the literature and critical reviews of the work which has been done. Investigators in the field of nutrition will find that this volume not only helps in becoming familiar with the literature on the various subjects discussed but that it also will be an aid in separating the wheat from the chaff. It is hoped that future volumes will be written in a similar vein. There are surprisingly few typographic errors. J. J.

ALLERGY. By ERICH URBACH, M.D., Chief of Allergy Service, Jewish Hospital, Philadelphia; Associate in Dermatology, University of Pennsylvania School of Medicine; and PHILIP M. GOTTLIEB, M.D., Associate on Allergy Service, Jewish Hospital, Philadelphia; Instructor in Medicine, University of Pennsylvania School of Medicine. Pp. 1100; 40 illus; 80 tables and charts. New York: Grune & Stratton, Inc., 1943. Price, $12.00.

A VAST increase in our knowledge of human hypersensitivity has accumulated in the past 15 years. Much of this is controversial or conjectural; thus, a text in its relatively new field is of timely interest.

The major part of the book has been written essentially for the clinician, particularly the internist. The subject matter is well arranged and well presented. Part I presents the basic science of allergy—written as an orientation and as reference material. Part II deals with the common etiologic agents, but omits the technical details such as botanical history of the pollen producers, etc. In Part III the author discusses the various diseases from the clinical and therapeutic points of view. In this section a chapter is given to the manifestations of allergy in each system, that is, Upper Respiratory Tract, Lower Respiratory Tract, Gastro Intestinal System, Liver and Gall Bladder, Skin, Nervous System, Eye, etc. The two final chapters deal with allergy in the very young, and in the aged—a welcome inclusion for those wishing to add to their repertory of geriatric principles. Graphic calendars of seasonal polination periods, diet charts, regimen outlines, testing media, and indicated medication provide an array of practical tools for the allergist. Of particular value is the replete system of cross-references which the neophyte allergist should find helpful in finding his way among the new terms introduced. The index is complete, and, with the reference system, the text can be used as an aid in finding the cause and treatment of an allergic disorder. The section devoted to asthma (110 pages) approaches monographic proportions. The reference sources, although chiefly English and American, include many in foreign languages. While numerous, the references are select; they are listed at the bottom of each page. Dr. Urbach expresses his own opinion freely, particularly on controversial material, and includes a minimum of information that must, to date, be classed as speculative. The author tends to dispel certain prevalent misconceptions, notably the inadequacy of skin

testing (which explains the disrepute into which this practice is falling). The truly remarkable specificity of the antigen-antibody reaction, the limitations of climatotherapy and the distinction between local hypersensitivity and the ability of the contactant to act as a primary irritant are topics worthy of note. The text should prove of great practical value to those with general or special interest in this field, and is heartily recommended. E. W.

MEMOIR OF WALTER REED. The Yellow Fever Episode. By ALBERT E. TRUBY, Brigadier General, U. S. A., Retired. Pp. 239; 29 figs. New York and London: Paul B. Hoeber, Inc., Medical Book Department of Harper & Brothers, 1943. Price, $3.50.

IN his earnestness to present all the documentary and factual data pertaining to the discovery of the cure and prevention of yellow fever, the author, for some at least, has sacrificed reader-interest. Without a doubt the yellow fever episode is one of intense dramatic interest—happening as it has within the lifetime of many of us. An untold amount of material has been written about different phases of the study; and naturally distortion of facts has crept into some of these writings. Thus, the purpose of the author has been to "clear up all doubtful or disputed points, to evaluate, as far as possible, the part played by each of the chief actors and to make an authentic and factual history of the great demonstration." Inasmuch as Brigadier General Truby was one of the main actors in this drama, he is well qualified to follow out his objective.

There is no doubt in the mind of the Reviewer that the author has set forth the pertinent facts with great accuracy and painstaking detail. It is merely unfortunate that such painstaking detail should detract from the vividness of the characters portrayed, so that they tend to become names on a page and fail to stand forth as human beings. Even though the great personality of the book, Walter Reed, is talked about from almost the first page—one still does not have a clear conception of Reed the Man. His reputation as a scientist was established before his untimely and early death from appendicitis, so that this book is not concerned with building him up from that angle—but we still do not actually know the human side of the great scientist, and this would have added much to the interest of the book. Yet we must not lose sight of the fact that such a book as this should be written, so that as history attains more of a perspective on this medical epoch, true facts will not become distorted or lost as the active participants disappear from the scene. E. F.

NEW BOOKS

Allergy. By ERICH URBACH, M.D., Chief of Allergy Service, Jewish Hospital, Philadelphia; Associate in Dermatology, University of Pennsylvania School of Medicine; and PHILIP M. GOTTLIEB, M.D., Associate on Allergy Service, Jewish Hospital, Philadelphia; Instructor in Medicine, University of Pennsylvania School of Medicine. Pp. 1100; 400 illus., 80 tables and charts. New York: Grune & Stratton, Inc., 1943. Price, $12.00. (Reviewed, p. 417.)

Annual Review of Biochemistry. Vol. XII. Edited by JAMES MURRAY LUCK, Stanford University; Associate Editor JAMES H. C. SMITH, Carnegie Institution of Washington, Division of Plant Biology, Stanford University, California. Pp. 704; many tables and figs. Stanford University P. O., Calif.: Annual Reviews, Inc., 1943. Price, $5.00.

Dictionary of Bio-chemistry and Related Subjects. Editor-in-Chief, WILLIAM M. MALISOFF, Professor of Bio-chemistry at the Polytechnic Institute of Brooklyn. Pp. 579. New York: Philosophical Library, Inc., 1943. Price, $7.50.

Borderlands of Psychiatry. By STANLEY COBB, Bullard Professor of Neuropathology, Harvard Medical School; Psychiatrist-in-Chief, Massachusetts General Hospital. Harvard University Monograph in Medicine and Public Health, #4. Pp. 166; 28 figs. Cambridge, Mass.: Harvard University Press, 1943. Price, $2.50.

A Synopsis of Clinical Syphilis. By JAMES KIRBY HOWLES, B.S., M.D., M.M.S., Professor of Dermatology and Syphilology, and Director of the Department, Louisiana State University School of Medicine; Senior Visiting Physician, Charity Hospital of Louisiana at New Orleans; Visiting Physician, French Hospital, Mercy Hospital, Hotel Dieu, Southern Baptist Hospital and Touro Infirmary. Pp. 761; 121 illus., 2 color plates. St. Louis: C. V. Mosby Company, 1943. Price, $6.00.

Memoir of Walter Reed. The Yellow Fever Episode. By ALBERT E. TRUBY, Brigadier General, United States Army, Retired. Pp. 239; 29 figs. New York, London: Paul B. Hoeber, Inc., Medical Book Department of Harper & Bros., 1943. Price, $3.50. (See Review, p. 418, this issue.)

Rehabilitation of the War Injured. Edited by WILLIAM BROWN DOHERTY, M.D., and DAGOBERT D. RUNES, PH.D. Pp. 684; many figs. New York: Philosophical Library, 1943. Price, $10.00.

The Boy Sex Offender and His Later Career. By LEWIS J. DOSHAY, M.D., PH.D., Psychiatrist, Children's Courts, New York City; formerly Senior Assistant Physician, Manhattan State Hospital, and Attending Specialist in Neuropsychiatry, U. S. Veterans Hospital, New York City. Pp. 248; 40 tables, 12 diagrams. New York: Grune & Stratton, 1943. Price, $3.50.

The Microscope and Its Use. By FRANK J. MUNOZ, Technical Microscope Consultant; in Collaboration with DR. HARRY A. CHARIPPER, Professor of Biology, New York University. Pp. 334; 122 figs. Brooklyn, N. Y.: Chemical Publishing Company, Inc., 1943. Price, $2.50.

Cytology and Cell Physiology. Edited by GEOFFREY BOURNE. Pp. 296; several figs. and plates. New York: Oxford University Press, 1942. Price, $6.00.

Medical Clinics of North America. Symposium on Physical Therapy. Mayo Clinic Number. Pp. 317. Philadelphia: W. B. Saunders Company, 1943. Price, $16.00 per year.

Addendum to the Chemistry of the Amino Acids and Proteins. Edited by CARL L. A. SCHMIDT, M.S., PH.D., Professor of Biochemistry and Dean of the College of Pharmacy, University of California. This is a supplement to the Second edition. Pp. 155; figures and tables. Springfield, Ill.: and Baltimore, Md.: Charles C Thomas, 1943. Price, $5.00.

NEW EDITIONS

Methods of Treatment. By LOGAN CLENDENING, M.D., Clinical Professor of Medicine, Medical Department of the University of Kansas; Attending Physician, University of Kansas Hospitals; and EDWARD H. HASHINGER, A.B., M.D., Clinical Professor of Medicine, Medical Department of the University of Kansas; Attending Physician, University of Kansas Hospitals; Attending Physician, St. Luke's Hospital, Kansas City, Mo. With chapters on Special Subjects. Eighth ed. Pp. 1033; 138 figs. St. Louis: C. V. Mosby Company, 1943. Price, $10.00. (See Review, p. 414, this issue.)

Clinical Parasitology. By CHARLES F. CRAIG, M.D., M.A. (Hon.), F.A.C.S., F.A.C.P., Col., U. S. Army (Retired), D.S.M., Emeritus Professor of Tropical Medicine in The Tulane University of Louisiana, New Orleans; and ERNEST C. FAUST, M.A., PH.D., Professor of Parasitology in the Department of Tropical Medicine, The Tulane University of Louisiana. Third ed. Pp. 767; 284 illus., 4 colored plates. Philadelphia: Lea & Febiger, 1943. Price, $9.00.

Roentgenographic Technique. By DARMON A. RHINEHART, A.M., M.D., F.A.C.R., Professor of Roentgenology and Applied Anatomy, School of Medicine, University of Arkansas; Roentgenologist to St. Vincent's Infirmary, Missouri Pacific Hospital, and the Arkansas Children's Hospital, Little Rock. Third ed. Pp. 471; 201 engrav. Philadelphia: Lea & Febiger, 1943. Price, $5.50.

A Workbook of Elementary Pharmacology and Therapeutics. By LUELLA C. SMITH, M.D., B.S. Second ed. Pp. 300. St. Louis: C. V. Mosby Company, 1943. Price, $2.00.

THIS loose-leaf book is primarily an extensive review of mathematics for nurses with emphasis upon the preparation of solutions and hypodermic doses and upon conversion from metric to apothecaries system. Some practical information concerning the administration of drugs is included in the last third of the book, but this can scarcely be called pharmacology in the modern sense. It is worth noting that the major portion of the book would be unnecessary if the medical and nursing professions would adopt the metric system. J. C.

The Modern Treatment of Syphilis. By JOSEPH EARLE MOORE, M.D., Associate Professor of Medicine and Adjunct Professor of Public Health Administration, The Johns Hopkins University; Physician-in-Charge, Syphilis Division of the Medical Clinic, and Visiting Physician, The Johns Hopkins Hospital, Baltimore; Special Consultant, United States Public Health Service. With the Collaboration of JAROLD E. KEMP, M.D., HARRY EAGLE, M.D., APUL PADGET, M.D., MARY S. GOODWIN, M.D., FRANK REYNOLDS, M.D., of whom all are members of the faculty of the Johns Hopkins University. Second ed. Pp. 717; 109 figs. Springfield, Ill., and Baltimore, Md.: Charles C Thomas, 1943. Price, $7.00.

THIS fresh version is a reprint of the first 32 chapters of the 2d edition without changes, while Chapter 33 on "Intensive Arsenotherapy of Early Syphilis" is completely rewritten and brought up-to-date and there is an addition of a 34th chapter on "Venereal Disease Control in the Army and Navy." Since a critical review of the first 32 chapters has recently appeared in this Journal (**204**, 120, 1942) it remains only to mention that Moore and his associates have provided a fruitful source of reference material for two pressing problems in today's Syphilology. H. B.

CORRECTION

In the Progress article by Mary Benson Hall on "Nausea and Vomiting of Pregnancy" appearing in the June number of this Journal, p. 872, Reference 56 (Strauss, M. B.) should be changed to 36 (Luikart, R.).

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